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PATENT APPLICATION

METHODS OF MODULATING PROLIFERATIVE CONDITIONS

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METHODS OF MODULATING PROLIFERATIVE CONDITIONS

[0001] This application claims benefit from U.S. Provisional Patent Application No. 60/398,088, filed July 24, 2002.

FIELD OF THE INVENTION

[0002] The present invention discloses methods for the diagnosis and treatment of proliferative conditions, e.g., cancer. In particular, it provides identification of a group of Myc-binding genes, and methods of using agonists or antagonists that modulate the activities of these genes and their gene products.

BACKGROUND OF THE INVENTION

[0003] Cancer of the gastrointestinal tract, pancreas, liver, prostate, breast, and the leukemias, are among the most frequent types of cancer (Tichopoulos, et al. (1997) in *Epidemiology of Cancer in Cancer: Principles and Practice of Oncology, Fifth Ed.*, ed. by DeVita, *et al.*, Lippincott-Raven Publishers, Phila., PA, pp. 231-257). Cancer develops in stages from normal cells to benign lesions, to malignant tumors such as carcinomas, and finally to invasive metastatic disease. Alterations in gene structure or gene expression appear to be responsible for the progression of cancer.

[0004] Nearly all cells in the body grow and divide, with well regulated periods of quiescence. These quiescent periods are markedly decreased or improperly regulated in cancer cells. Cell proliferation, i.e., the rate of cell division, is controlled by genes that regulate the rate of cell growth, division, and quiescence. In addition to increased cell proliferation, cancer is distinguished by changes in the regulation of genes that control angiogenesis and metastasis. Cells divide or remain quiescent as a result of certain proteins that function to regulate intracellular messages. Some of these proteins bind to DNA and regulate gene activity by binding DNA, while others are membrane-bound or remain free in solution. Examples of

signaling proteins that bind to DNA include, e.g., Myc, Ras, Jun, and Fos. Examples of signaling proteins that do not bind to DNA include, e.g., cyclins and certain protein kinases.

[0005] Myc gene is closely associated with the etiology of cancer, as mutations or changes in intracellular levels of Myc occur in various cancers. Myc protein has been implicated in the regulation of a number of genes. The identification of which of these putative Myc-regulated genes are important to cancer has been difficult. The present invention provides a solution to this problem by disclosing a group of genes comprising regulatory regions that bind Myc, *in vivo*.

SUMMARY OF THE INVENTION

[0006] The present invention is based, in part, upon the discovery of a group of Myc-binding genes that can affect cell proliferation and cancer.

[0007] The invention provides a method of regulating cell proliferation comprising modulating the activity of a gene or polypeptide of Table 2, the above method wherein the gene is positive for Myc binding in a chromatin immunoprecipitation (ChIP) assay, the above method wherein the modulating is inhibiting or activating, and the above method wherein the cell proliferation is oncogenic.

[0008] Also provided is a method of regulating cell proliferation comprising modulating the activity of gene or polypeptide of Table 2, wherein the modulating is by a binding composition, or wherein the binding composition comprises an antigen-binding site of an antibody, a soluble receptor, a nucleic acid, or a small molecule, or wherein the binding composition comprises a human or humanized antibody; a monoclonal antibody; a polyclonal antibody; an Fab fragment or an $F(ab')_2$ fragment; a peptide mimetic of an antibody; a detectable label; or an anti-sense nucleic acid.

[0009] In another embodiment, the invention provides a method for the diagnosis of a proliferative condition comprising detecting or determining the expression or activity of at least one gene or polypeptide of Table 2, the above method wherein the gene is positive for Myc binding in a ChIP assay, the above method wherein the detecting or determining is by a binding composition comprising the antigen binding site from an antibody, a soluble receptor, or a

nucleic acid, and the above method wherein the binding composition comprises a human or humanized antibody; a monoclonal antibody; a polyclonal antibody; an Fab fragment or an $F(ab')_2$ fragment; a peptide mimetic of an antibody; a detectable label; or a nucleic acid probe or nucleic acid primer.

[0010] Yet another aspect of the present invention is a method of treating a subject suffering from a proliferative disorder comprising administering to the subject an effective amount of an agonist or antagonist of at least one gene or polypeptide of Table 2, the above method wherein the gene is positive for Myc binding in a ChIP assay, and the above method wherein the proliferative disorder is oncogenic. The contemplated invention encompasses a method of treating a subject suffering from a proliferative disorder comprising administering to the subject an effective amount of an agonist or antagonist of at least one gene or polypeptide of Table 2, wherein the treating is by a binding composition, the above method wherein the binding composition comprises an antigen-binding site of an antibody, a soluble receptor, a nucleic acid, or a small molecule, and the above method wherein the binding composition comprises a human or humanized antibody; a monoclonal antibody; a polyclonal antibody; an Fab fragment or an $F(ab')_2$ fragment; a peptide mimetic of an antibody; a detectable label; or an anti-sense nucleic acid.

DETAILED DESCRIPTION

[0011] As used herein, including the appended claims, the singular forms of words such as "a," "an," and "the" include their corresponding plural references unless the context clearly dictates otherwise.

[0012] All references cited herein are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

I. Definitions.

[0013] "Activity" of a molecule refers, e.g., to binding of the molecule to a ligand or to a receptor, to catalytic activity, to the ability to stimulate, maintain, or inhibit gene expression, to antigenic activity, to the modulation of activities of other molecules, to modulation of ion transport, and the like. "Activity" of a molecule may also refer to activity in modulating or maintaining cell-to-cell interactions, e.g., adhesion, or activity in maintaining a structure of a cell, e.g., cell membranes or cytoskeleton. "Activity" may also mean specific activity, e.g., [catalytic activity]/[mg protein], or [immunological activity]/[mg protein], or the like. Activity of a nucleic acid may refer to expression of a gene, e.g., rate of transcription from the gene, to rate of translation of an mRNA, or to concentration of the mRNA in a cell or tissue.

[0014] "Amino acid" refers to naturally occurring and synthetic amino acids, as well as to amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, including selenomethionine, as well as those amino acids that are modified after incorporation into a polypeptide, e.g., hydroxyproline, γ-carboxyglutamate, O-phosphoserine, and cystine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an alpha-carbon that is bound by a hydrogen, carboxyl group, amino group, and an R group. Amino acid analogs include, e.g., homoserine, norleucine, methionine sulfoxide, and methionine methyl sulfonium. "Amino acid mimetics" refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid. Amino

acids may be referred to herein by either their commonly known three letter symbols or by their one-letter symbols.

[0015] "Angiogenesis" is the growth of new blood vessels in a tissue or organism.

[0016] "Antibody" refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically recognizes and binds an antigen. The immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. A "partially humanized" or "chimeric" antibody contains heavy and light chain variable regions of, e.g., murine origin, joined onto human heavy and light chain constant regions. A "humanized" or "fully humanized" antibody contains the amino acid sequences from the six complementarity-determining regions (CDRs) of the parent antibody, e.g., a mouse antibody, grafted to a human antibody framework. "Human" antibodies are antibodies containing amino acid sequences that are of 100% human origin, where the antibodies may be expressed, e.g., in a human, animal, bacterial, or viral host (Baca, *et al.* (1997) *J. Biol. Chem.* 272:10678-10684; Clark (2000) *Immunol. Today* 21:397-402).

[0017] Antibodies exist, e.g., as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce $F(ab)'_2$, a dimer of Fab which itself is a light chain joined to V_H-C_H1 by a disulfide bond. The $F(ab)'_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the $F(ab)'_2$ dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region.

[0018] "Fv" fragment comprises a dimer of one heavy chain and one light chain variable domain in tight association with each other. A single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0019] "Monoclonal antibody" (mAb) refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibody polypeptides comprising

the population are identical except for possible naturally occurring mutations in the polypeptide chain that may be present in minor amounts. The term "monoclonal antibody" does not suggest any characteristic of the oligosaccharide component, or that there is homogeneity or heterogeneity with regard to oligosaccharide component. Monoclonal antibodies are highly specific, being directed against a single antigenic site or epitope. In contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different epitopes, each mAb is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they can be synthesized by hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. "Monoclonal antibodies" also include clones of antigen-recognition and binding-site containing antibody fragments, such as those derived from phage antibody libraries.

[0020] "Diabodies" refers to a fragment comprising a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) (Hollinger, *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448).

[0021] "Binding composition" refers to a molecule, small molecule, macromolecule, antibody, or a fragment or analogue thereof, or soluble receptor, capable of binding to a target. "Binding composition" also may refer to a complex of molecules, e.g., a non-covalent complex, to an ionized molecule, and to a covalently or non-covalently modified molecule, e.g., modified by phosphorylation, acylation, cross-linking, or cyclization, which is capable of binding to a target. "Binding composition" may also refer to a molecule in combination with a stabilizer, excipient, salt, buffer, solvent, or additive, capable of binding to a target. "Binding" may be defined as an association of the binding composition with a target where the association results in reduction in the normal Brownian motion of the binding composition, in cases where the binding composition can be dissolved or suspended in solution. "Modulating by a binding composition" can be effected by, e.g., treatment, administration, or contacting of a binding composition to a cell, host cell, cancer cell, tumor, tissue, organ, physiological fluid, research or clinical patient or animal. "Modulation" includes modulation of activity of, e.g., a gene, protein, polypeptide, or cellular function.

[0022] The phrase "specifically" or "selectively" binds, when referring to a ligand/receptor, antibody/antigen, or other binding pair, refers to a binding reaction which is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Under designated conditions, a specified ligand binds to a particular, e.g., protein, receptor, or antigen, and binds to a lesser extent to other, e.g., protein, receptor, or antigen. The contemplated ligand or antibody of the invention binds to its target, e.g., a receptor or antigen, or a variant or mutein of the target, with an affinity that is generally two-fold greater, more generally four-fold greater, preferably 10-times greater, and still more preferably 20-times greater than the binding affinity to any other potential target. In a preferred embodiment the ligand or antibody will have an affinity which is greater than about 10^9 liters/mol, as determined, e.g., by Scatchard analysis (Munsen, *et al.* (1980) *Analyt. Biochem.* 107:220-239).

[0023] "Cell line" refers to a population of cells capable of continuous or prolonged growth and division *in vitro*. Often, cell lines are clonal populations derived from a single progenitor cell. Spontaneous or induced changes can occur in the genome or can occur during storage or transfer of one or more cells present in the population of cells. Therefore, cells derived from the cell line referred to may not be precisely identical to the ancestral cells or cultures, and the cell line referred to includes such variants. The term "cell line" also includes immortalized cells (U.S. Patent No. 6,090,611 issued to Covacci, *et al.*).

[0024] "Cell proliferation" is the rate of increase in cell number and is a function of the rate of cell division. Depending on the context, "cell proliferation" may indicate an overall increase in cell number, which is a function of cell division, cell death, or cell removal. Alternatively, cell proliferation may be used to indicate a quantity reflecting solely the rate of cell division. Proliferation may encompass phenomena such as the cell cycle, nutrient transport, growth, apoptosis, angiogenesis, and cell differentiation, where the phenomenon in question contributes to an increase in the rate of cell division or an increase in cell number.

[0025] Administration "in combination with" one or more therapeutic agents includes simultaneous or concurrent administration and consecutive administration, in any order.

[0026] "Chromatin" is the complex of genomic nucleic acids and proteins that can be found in the nucleus of the living cell, or in the cytoplasm of the cell when the nuclear membrane disappears, e.g., in mitosis or meiosis. The bound proteins include histones, modified histones,

transcription factors, DNA polymerases, DNA repair proteins, and proteins controlling higher level structures of chromatin.

[0027] "Consensus E-boxes" and "non-consensus E-boxes" are defined (Blackwell, *et al.* (1993) *Mol. Cell. Biol.* 13:5216-5224; Grandori, *et al.* (1996) *EMBO J.* 15:4344-4357). The term E-box may refer to an E-box as it occurs in single stranded or in double stranded nucleic acids. Functional properties can provide guidance in defining E-boxes that vary somewhat from the consensus sequence, i.e., E-boxes classed as non-consensus or non-canonical E-boxes.

[0028] "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical nucleic acid sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a conserved amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant." Conservative substitution tables providing functionally similar amino acids are well known in the art. An example of a conservative substitution is the exchange of an amino acid in one of the following groups for another amino acid of the same group (U.S. Patent No. 5,767,063 issued to Lee, *et al.*; Kyte and Doolittle (1982) *J. Mol. Biol.* 157:105-132):

(1) Hydrophobic: Norleucine, Ile, Val, Leu, Phe, Cys, or Met;

(2) Neutral hydrophilic: Cys, Ser, Thr;

(3) Acidic: Asp, Glu;

(4) Basic: Asn, Gln, His, Lys, Arg;

(5) Residues that influence chain orientation: Gly, Pro;

(6) Aromatic: Trp, Tyr, Phe;

(7) Small amino acids: Gly, Ala, Ser.

[0029] "Detecting" generally relates to data that is or can be communicated or recorded as positive or negative, e.g., + or -, while "determining" generally relates to data that is or can be

communicated or recorded as positive or negative, or in graded quantities, e.g., as -, +, ++, and +++, or in numerical quantities.

[0030] "Exogenous" refers to substances that are produced outside a cell, tissue, or organism, depending on the context. "Endogenous" refers to substances that are produced within a cell, tissue, or organism, depending on the context.

[0031] An "expression vector" is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid. Typically, the expression vector includes a nucleic acid to be transcribed operably linked to a promoter.

[0032] "Gene expression" refers to transcription or translation, depending on the context. In transcription, mRNA is expressed from a gene. In translation, a polypeptide is expressed from mRNA.

[0033] An "immunoassay" is an assay that uses an antibody, antibody fragment, or antigen binding site derived from an antibody, to specifically bind an antigen. The immunoassay is characterized by the use of specific binding properties of a particular antibody to isolate, target, or quantify the antigen.

[0034] An "inhibitor" or "antagonist" refers, e.g., to a molecule, complex, or composition that reduces the activity of, e.g., a ligand, receptor, cofactor, nucleic acid, gene, cell, tissue or organ. An "activator" or "agonist" refers, e.g., to a molecule, complex, or composition that increases the activity of, e.g., a ligand, receptor, cofactor, nucleic acid, gene, cell, tissue or organ. "Modulator" refers to, e.g., a molecule, complex, or composition, that serves as an inhibitor or activator. The modulator can act alone, or it may use a cofactor, e.g., a protein, metal ion, or small molecule. Inhibitors are compounds that decrease, block, prevent, delay activation, inactivate, desensitize, or down regulate, e.g., a gene, protein, or cell. An inhibitor may also be defined as a composition that reduces, blocks, or inactivates a constitutive activity. Activators are compounds that increase, activate, facilitate, enhance activation, sensitize, or up regulate, e.g., a gene, protein, or cell. An "agonist" is a compound that interacts with a target to cause or promote an increase in the activation of the target. An "antagonist" is a compound that opposes the actions of an agonist. An antagonist prevents, reduces, inhibits, or neutralizes the activity of

an agonist. An antagonist can also prevent, inhibit, or reduce constitutive activity of a target, even where there is no identified agonist.

[0035] To examine the extent of inhibition, samples or assay mixtures comprising, e.g., a given nucleic acid, polypeptide, cell, tissue, or organism, are treated with a potential activator or potential inhibitor and are compared to control samples without the inhibitor. Control samples, i.e., not treated with antagonist, are assigned a relative activity value of 100%. Inhibition is achieved when the activity value relative to the control is about 90% or less, typically 85% or less, more typically 80% or less, most typically 75% or less, generally 70% or less, more generally 65% or less, most generally 60% or less, typically 55% or less, usually 50% or less, more usually 45% or less, most usually 40% or less, preferably 35% or less, more preferably 30% or less, still more preferably 25% or less, and most preferably 25% or less. Activation is achieved when the activity value relative to the control is about 110%, generally 120%, more generally 140%, more generally at least 160%, often 180%, more often 2-fold, most often 2.5-fold, usually 5-fold, more usually 10-fold, preferably 20-fold, more preferably 40-fold, and most preferably over 40-fold higher.

[0036] "Detectable inhibition" or "detectable decrease," e.g., in expression of a gene or polypeptide of Tables 1 or 2, or of a predetermined activity, refers, e.g., to a comparison of expression or activity in the presence and absence of an agonist of a gene or polypeptide of Tables 1 or 2, or in the presence or absence of an antagonist of a gene or polypeptide of Tables 1 or 2. "Detectable" may be a function of the context, e.g., of the reagents, instrumentation, or biological system. "Activity of a gene" may be defined as a rate, e.g., the rate of transcription, rate of translation, or as a concentration, e.g., concentration of the transcription or translation product in a cell, tissue, extract, or isolate.

[0037] Endpoints in activation or inhibition can be monitored as follows. Activation, inhibition, and response to treatment, of a cell, physiological fluid, tissue, organ, and animal or human subject, can be monitored by an endpoint. The endpoint may comprise a predetermined quantity or percentage of cell degranulation or secretion, e.g., of a cytokine, toxic oxygen, or a protease. Alternatively, the endpoint may comprise a predetermined quantity of ion flux, e.g., calcium flux, cell migration, cell adhesion, cell proliferation, potential for metastasis, cell differentiation, and change in phenotype, e.g., change in expression of gene relating to

inflammation, apoptosis, transformation, cell cycle, or metastasis, see, e.g., Knight (2000) *Ann. Clin. Lab. Sci.* 30:145-158; Hood and Cheresh (2002) *Nature Rev. Cancer* 2:91-100; Timme, *et al.* (2003) *Curr. Drug Targets* 4:251-261; Robbins and Itzkowitz (2002) *Med. Clin. North Am.* 86:1467-1495; Grady and Markowitz (2002) *Annu. Rev. Genomics Hum. Genet.* 3:101-128; Bauer, *et al.* (2001) *Glia* 36:235-243; Stanimirovic and Satoh (2000) *Brain Pathol.* 10:113-126). Generally, the endpoint of inhibition is 75% or less than the control, preferably the endpoint is 50% or less than the control, more preferably the endpoint is 25% or less than the control, and most preferably the endpoint is 10% or less than the control. Generally, the endpoint of activation is at least 150% control, preferably the endpoint is at least two times the control, more preferably the endpoint is at least four times the control, and most preferably the endpoint is at least 10 times the control.

[0038] A composition that is "labeled" is detectable, either directly or indirectly, by spectroscopic, photochemical, biochemical, immunochemical, isotopic, or chemical means. For example, useful labels include ^{32}P, ^{33}P, ^{35}S, ^{14}C, ^{3}H, ^{125}I, stable isotopes, fluorescent dyes and fluorettes (Rozinov and Nolan (1998) *Chem. Biol.* 5:713-728; Molecular Probes, Inc. (2003) *Catalogue*, Molecular Probes, Eugene OR), electron-dense reagents, enzymes and/or substrates, e.g., as used in enzyme-linked immunoassays as with those using alkaline phosphatase or horse radish peroxidase. The label or detectable moiety is typically bound, either covalently, through a linker or chemical bound, or through ionic, van der Waals or hydrogen bonds to the molecule to be detected. "Radiolabeled" refers to a compound to which a radioisotope has been attached through covalent or non-covalent means. A "fluorophore" is a compound or moiety that absorbs radiant energy of one wavelength and emits radiant energy of a second, longer wavelength.

[0039] A "labeled nucleic acid probe or oligonucleotide" is one that is bound, either covalently, through a linker or a chemical bond, or noncovalently, through ionic, van der Waals, electrostatic, or hydrogen bonds to a label such that the presence of the probe can be detected by detecting the presence of the label bound to the probe. The probes are preferably directly labeled as with isotopes, chromophores, fluorophores, chromogens, or indirectly labeled such as with biotin to which a streptavidin complex or avidin complex can later bind.

[0040] "Ligand" refers to an entity that specifically binds to a polypeptide, to a complex comprising more than one polypeptide, or to a macromolecule such as a nucleic acid. A "ligand

binding domain" or receptor is a region of, e.g., a polypeptide or nucleic acid, that is able to bind the ligand. A ligand can comprise, e.g., a soluble protein, membrane-associated protein, integral membrane-bound protein, oligosaccharide, lipid, or nucleic acid. Where a ligand binds to a receptor, the question of which molecule is the ligand and which molecule is the receptor can be determined on a case-by-case basis. Generally, where the binding event results in cell signaling, a molecule that is constitutively bound to the cell that responds to the signal may be considered to be part of the receptor, and not part of the ligand. A freely diffusable and water-soluble entity that is involved in ligand/receptor interactions is usually a ligand, not a receptor.

[0041] "Metastasis" is the process where a primary tumor mass spawns pioneer cells that invade adjacent tissues and travel to distant sites, where they found new colonies (Hanahan and Weinberg (2000) *Cell* 100:57-70).

[0042] "Myc" refers to a family of genes and corresponding polypeptides. The Myc family includes c-Myc, N-Myc, L-Myc, S-Myc, and B-Myc. These proteins are most closely homologous at the MB1 and MB2 regions in the N-terminal region and at the basic helix-loop-helix leucine zipper (bHLHLZ) motif in the C-terminal region (Oster, *et al.* (2002) *Adv. Cancer Res.* 84:81-154; Grandori, *et al.*(2000) *Annu. Rev. Cell Dev. Biol.* 16:653-699). Myc also encompasses versions of Myc that are non, partially, and fully phosphorylated.

[0043] "Nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof, including single stranded and double stranded forms. The term encompasses nucleic acids containing nucleotide analogs or modified backbone residues or linkages. Examples of such analogs, e.g., phosphorothioates, phosphoramidates, and peptide-nucleic acids (PNAs).

[0044] A particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof, e.g., degenerate codon substitutions, and complementary sequences. "Nucleic acid" may be used to refer, e.g., to a gene, cDNA, mRNA, oligonucleotide, or polynucleotide. A particular nucleic acid sequence also implicitly encompasses, e.g., allelic variants, splice variants, and muteins.

[0045] "Nucleic acid probe" is a nucleic acid capable of binding to a target nucleic acid of complementary sequence, usually through complementary base pairing, e.g., through hydrogen bond formation. A probe may include natural, e.g., A, G, C, or T, or modified bases, e.g., 7-deazaguanosine, inosine, etc. The bases in a probe can be joined by a linkage other than a

phosphodiester bond. Probes can be peptide nucleic acids in which the constituent bases are joined by peptide bonds rather than phosphodiester linkages. It will be understood by one of skill in the art that probes may bind target sequences lacking complete complementarity with the probe sequence depending upon the stringency of the hybridization conditions.

[0046] "Polymerase chain reaction" (PCR) refers, e.g., to a procedure or product where a specific region or segment of a nucleic acid is amplified, and where the segment is bracketed by primers used by DNA polymerase (Bernard and Wittwer (2002) *Clin. Chem.* 48:1178-1185; Joyce (2002) *Methods Mol. Biol.* 193:83-92; Ong and Irvine (2002) *Hematol.* 7:59-67).

[0047] A "promoter" is a nucleic acid sequence that directs transcription of a nucleic acid. A promoter includes nucleic acid sequences near the start site of transcription, e.g., a TATA box, see, e.g., Butler and Kadonaga (2002) *Genes Dev.* 16:2583-2592; Georgel (2002) *Biochem. Cell Biol.* 80:295-300. A promoter also optionally includes distal enhancer or repressor elements, which can be located as much as several thousand base pairs on either side from the start site of transcription. A "constitutive" promoter is a promoter that is active under most environmental and developmental conditions, while an "inducible" promoter is a promoter is active or activated under, e.g., specific environmental or developmental conditions. "Association of an E box with a promoter" means, e.g., that binding of Myc to the E box results in a change in gene expression from that promoter, where the change may comprise, e.g., an increase or a decrease in the rate of gene expression.

[0048] "Protein" generally refers to the sequence of amino acids comprising a polypeptide chain. Protein may also refer to a three dimensional structure of the polypeptide. "Denatured protein" refers to a partially denatured polypeptide, having some residual three dimensional structure or, alternatively, to an essentially random three dimensional structure, i.e., totally denatured. The invention encompasses variants of proteins, and relevant methods, involving, e.g., glycosylation, phosphorylation, sulfation, disulfide bond formation, deamidation, isomerization, cleavage points in signal or leader sequence processing, covalent and non-covalently bound cofactors, oxidized variants, alternate folding, and the like. Disulfide links are described, e.g., see Woycechowsky and Raines (2000) *Curr. Opin. Chem. Biol.* 4:533-539; Creighton, *et al.* (1995) *Trends Biotechnol.* 13:18-23.

[0049] By "purified" and "isolated" is meant, when referring to a polypeptide, that the polypeptide is present in the substantial absence of the other biological macromolecules. The term "purified" as used herein means typically about 70%, more typically 75%, at least 80%, ordinarily 85%, more ordinarily 90%, preferably 95%, and more preferably 98% by weight, or greater, of biological macromolecules present. The weights of water, buffers, salts, detergents, reductants, protease inhibitors, stabilizers, excipients, and other small molecules, especially those having a molecular weight of less than 1000, are generally not used in the determination of polypeptide purity (U.S. Patent No. 6,090,611). Purity and homogeneity are typically determined using methods well known in the art (Scopes (1994) *Protein Purification: Principles and Practice*, Springer-Verlag, NY, NY; Cunico, Gooding, and Wehr (1998) *Basic HPLC and CE of Biomolecules*, Bay Biological Laboratory, Inc. Hercules, CA).

[0050] "Recombinant" when used with reference, e.g., to a nucleic acid, cell, virus, plasmid, vector, or the like, indicates that these have been modified by the introduction of an exogenous, non-native nucleic acid or the alteration of a native nucleic acid, or have been derived from a recombinant nucleic acid, cell, virus, plasmid, or vector. Recombinant protein refers to a protein derived from a recombinant nucleic acid, virus, plasmid, vector, or the like.

[0051] "Small molecule" is defined as a molecule with a molecular weight that is less than 10 kD, typically less than 2 kD, and preferably less than 1 kD. Small molecules include, but are not limited to, inorganic molecules, organic molecules, organic molecules containing an inorganic component, molecules comprising a radioactive atom, synthetic molecules, peptide mimetics, and antibody mimetics. As a therapeutic, a small molecule may be more permeable to cells, less susceptible to degradation, and less apt to elicit an immune response than large molecules. Small molecule toxins are described, see, e.g., U.S. Patent No. 6,326,482 issued to Stewart, *et al.*

[0052] "Soluble receptor" refers to receptors that are water-soluble and occur, e.g., in extracellular fluids, intracellular fluids, or weakly associated with a membrane. Soluble receptor also refers to receptors that are released from tight association with a membrane, e.g., by limited cleavage. Soluble receptor further refers to receptors that are engineered to be water soluble, see, e.g., Monahan, *et al.* (1997) *J. Immunol.* 159:4024-4034; Moreland, *et al.* (1997) *New Engl. J.*

Med. 337:141-147; Borish, *et al.* (1999) *Am. J. Respir. Crit. Care Med.* 160:1816-1823; Uchibayashi, *et al.* (1989) *J. Immunol.* 142:3901-3908.

[0053] "Treatment" refers to both therapeutic treatment and prophylactic or preventative measures.

II. General.

[0054] Myc is a transcription factor that binds to a specialized transcription regulation sequence known as an E-box, often resulting in increased gene expression. Deletion of E-boxes can result in decreased gene expression (Greasley, *et al.* (2000) *Nucleic Acids Res.* 28:446-453). Myc binds to a target gene by way of one or more E-boxes associated with that gene. However, no single target of Myc seems to account fully for Myc's biological effects, as several Myc targets appear to cooperate to maintain normal physiology, or to create cell transformation when Myc is overexpressed (Levens (2002) *Proc. Natl. Acad. Sci. USA* 99:5757-5759).

[0055] Myc plays a role in regulating cell proliferation, the cell cycle, cell growth, angiogenesis, apoptosis, and oncogenesis. Myc's activity can increase in tumors as a consequence of mutations, chromosomal rearrangements, increased expression, or gene amplification, e.g., see Nesbit, *et al.* (1999) *Oncogene* 18:3004-3016; Zeller, *et al.* (2001) *J. Biol. Chem.* 276:48285-48291; He, *et al.* (1998) *Science* 281:1509-1512; McMahon, *et al.* (1998) *Cell* 94:363-374; Erisman, *et al.* (1985) *Mol. Cell. Biol.* 5:1969-1976; Rochlitz, *et al.* (1996) *Oncology* 53:448-454. Elevated Myc activity in cancer cells may be a consequence of mutations in oncogenes other than Myc, e.g., APC or β-catenin (He, *et al.* (1998) *supra*). Increased Myc levels have been documented, e.g., in breast cancer and prostate cancer (Liao and Dickson (2000) *Endocrine-Related Cancer* 7:143-164; Jenkins, *et al.* (1997) *Cancer Res.* 57:524-531).

[0056] Myc regulates the cell cycle, growth, and apoptosis. Changes in cell cycle regulation can result in increased cell proliferation. When Myc regulates the cell cycle, it can act as a signaling agent that promotes entry of a cell into the cell cycle (Trumpp, *et al.* (2001) *Nature* 414:768-773; Holzel, *et al.* (2001) *EMBO Reports* 21:1125-1132; Bouchard, *et al.* (2001) *Genes Devel.* 15:2042-2047). Myc has been found to act in specific phases of the cell cycle, where certain cell cycle genes, e.g., cyclins and protein kinases, are directly or indirectly regulated by Myc (Oster, *et al.*, *supra*). The invention provides methods for modulating the cell cycle.

[0057] Myc regulates growth, as it plays a role in regulating genes required for protein synthesis, e.g., genes for transcription factors and ribosomal proteins (Greasley, *et al.* (2000) *supra*; Zeller, *et al.* (2001) *supra*; Menssen and Hermeking (2002) *Proc. Natl. Acad. Sci. USA* 99:6274-6279). The invention contemplates methods for modulating growth.

[0058] Myc regulates apoptosis. Apoptosis can be impaired in cancer cells, as these cells are often able to avoid removal by cells of the immune system, survive in new locations in the body, or resist chemotherapy (Reed (2002) *Apoptosis* in *The Cancer Handbook* (Ed. by M.R. Alison) Nature Publishing Group, London, pp. 119-134). Myc regulates key apoptosis pathway proteins (Nesbit, *et al.* (1998) *Blood* 92:1003-1010; Oster, *et al.* (2002) *supra*). The contemplated invention provides methods for modulating apoptosis.

[0059] Techniques sensitive to the *in* vivo binding of Myc to candidate genes can identify Myc-target sites, as well as intracellular or extracellular factors that control Myc binding to these candidate genes. The invention identifies E-box containing Myc-target genes and polypeptides, and provides methods for modulating expression and activity of these genes and polypeptides for the treatment of abnormal or pathologic cell proliferation, cell growth, metastasis, angiogenesis, and apoptosis (Pelangaris, *et al.* (2000) *Curr. Opin. Genet. Dev.* 10:100-105). Provided are methods of modulating expression or activity of a nucleic acids or polypeptide of Tables 1 and 2, as well as methods of diagnosing disorders or pathological conditions associated with a nucleic acid or polypeptide of Tables 1 and 2. These nucleic acids and polypeptides include, e.g., CLCN6, SLC4A2, CLNS1A, TAPK, and netrin-2 like protein.

[0060] CLCN6, SLC4A2, CLNS1A, and TAPK are ion transporters or ion channels (Tables 1 and 2). Ion transporters can modulate cell proliferation, apoptosis, and metastasis. Change in intracellular pH, e.g., alkalinization, is a common feature of proliferating cells and tumor cells, where pH change results from changes in ion transporter activity. Ion transporter activity can serve as a checkpoint in the cell cycle. Chloride transporters can stimulate proliferation or cell invasiveness, as shown, e.g., by studies with chloride channel inhibitors. An additional role of ion transporters and cancer is function in extruding anti-cancer drugs, see, e.g., Elble and Pauli (2001) *J. Biol. Chem.* 276:40510-40517; Szabo, *et al.* (1998) *Proc. Natl. Acad. Sci. USA* 95:6169-6174; Bustin, *et al.* (2001) *DNA and Cell Biology* 20:331-338; Soroceanu, *et al.* (1999) *J. Neuroscience* 19:5942-5954; Abdel-Ghany, *et al.* (2001) *J. Biol. Chem.* 276:25438-

25446; Reshkin, *et al.* (2000) *FASEB J.* 2185-2197; Chien, *et al.* (2001) *J. Cellular Biochem.* 81:604-612; Wang, *et al.* (2002) *J. Cellular Physiol.* 193:110-119; Ransom, *et al.* (2001) *J. Neurosci.* 21:7674-7683; Bustin, *et al.* (2001) *DNA and Cell Biol.* 20:331-338; Schlichter, *et al.* (1996) *Glia* 17:225-236; Blaisdell, *et al.* (1999) *Am. J. Respir. Cell Mol. Biol.* 20:842-847; Shen, *et al.* (2000) *J. Physiol. (London)* 529:385-394; Pappas and Ritchie (1998) *Glia* 22:113-120; Martinez-Zaguilan, *et al.* (1999) *Biochem. Pharmacol.* 57:1037-1046.

[0061] CLCN6 (Tables 1 and 2) is a chloride channel (Kornak, *et al.* (1999) *Biochim. Biophys. Acta* 1447:100-106). CLCN6 occurs near a position of the chromosome that is often deleted in cancer, e.g., ovarian, breast, colorectal cancer, and neuroblastoma (Gaughan, *et al.* (2000) *Gene* 257:279-289). The invention contemplates use of CLCN6 polypeptides and nucleic acids, antigenic fragments thereof, and binding compositions specific for CLCN6 polypeptides and nucleic acids, for the treatment and diagnosis of proliferative disorders, e.g., cancer.

[0062] SLC4A2 (Tables 1 and 2) is a chloride/bicarbonate anion exchanger (Lecanda, *et al.* (2000) *Biochem. Biophys. Res. Commun.* 276:117-124; Medina, *et al.* (2000) *Biochem. Biophys. Res. Commun.* 276:228-235; Medina, *et al.* (1997) *Genomics* 39:74-85; Karet, *et al.* (1999) *Am. J. Hum. Genet.* 65:1656-1665). Cl^-/HCO_3^- exchangers modulate intracellular pH. The invention contemplates use of SLC4A2 polypeptides and nucleic acids, antigenic fragments thereof, and binding compositions specific for SLC4A2 polypeptides and nucleic acids, for the treatment and diagnosis of proliferative disorders, e.g., cancer.

[0063] CLNS1A (a.k.a. Icln; I_{cln}) (Tables 1 and 2) is a chloride transporter (Nagl, *et al.* (1996) *Genomics* 38:438-441; Scandella, *et al.* (2000) *J. Biol. Chem.* 275:15613-15620; Emma, *et al.* (2000) *Am. J. Physiol.* 274:C1545-C1551). CLNS1A resides on a region of the genome that is amplified in a subset of breast carcinomas prone to metastasis (Bekri, *et al.* (1997) *Cytogenet. Cell Genet.* (1997) 79:125-131). CLNS1A interacts with a protein (IBP72) that binds to a PAK-like kinase and appears to participate in cell cycling (Pu, *et al.* (2000) *J. Biol. Chem.* 275:12363-12366; Krapivinsky, *et al.* (1998) *J. Biol. Chem.* 273:10811-10814; Abe, *et al.* (1993) *Biochim. Biophys. Acta* 1173:353-356). The gene occurs in two locuses on the human genome, i.e., CLNS1A, which contains introns, and CLNS1B, which does not contain introns (Scandella, *et al., supra*). The E box of AF128461 occurs within an intron of human CLNS1A (GenBank NP_001284; P54105) (Tables 1 and 2). The invention contemplates use of CLNS1A

polypeptides and nucleic acids, antigenic fragments thereof, and binding compositions specific for CLNS1A polypeptides and nucleic acids, for the treatment and diagnosis of proliferative disorders, e.g., cancer.

[0064] Teratoma-associated tyrosine kinase (TAPK; gklp; ntkl) (Tables 1 and 2) resides in a region of the genome that contains breakpoints for chromosomal locations, where breakage occurs in various cancers. TAPK contains a protein kinase-like domain, but was found not to possess kinase activity. A mouse protein, p105, was found to be homologous to TAPK (van Asseldonk, *et al.* (2000) *Genomics* 66:35-42; Kato, *et al.* (2002) *Genomics* 79:760-767; Liu, *et al.* (2000) *Biochim. Biophys. Acta* 1517:148-152). The invention contemplates use of TAPK polypeptides and nucleic acids, antigenic fragments thereof, and binding compositions specific for TAPK polypeptides and nucleic acids, for the treatment and diagnosis of proliferative disorders, e.g., cancer.

[0065] Netrin-2 like protein (NTN2L) is a member of the netrin family of proteins, a family that includes netrin-1, nitrin-2, and netrin-3. The netrins, expressed by the nervous system, endocrine glands, muscle, and lungs, have been found to provide guidance to growing cells, e.g., axons, and to serve as a chemorepellent. NTN2L, a human netrin, is related to mouse netrin-3. Netrin-1 and netrin-3 bind to a number of receptors, e.g., DCC, neogenin, UNC5H1, UNC5H2, and UNC5H3 (Schuldt (2003) *Nature* 422:125; Guthrie (1997) *Current Biol.* 7:R6-R9; Wang, *et al.* (1999) *J. Neuroscience* 19:4938-4947; Livesey (1999) *Cell. Mol. Life Sci.* 56:62-68; Livesey and Hunt (1997) *Mol. Cell. Neurosci.* 8:417-429; Van Raay, *et al.* (1997) *Genomics* 41:279-282; Forcet, *et al.* (2002) *Nature* 417:443-447). DCC can mediate apoptosis or cell cycle arrest (Liu, *et al.* (2002) *J. Biol. Chem.* 277:26281-26285; Forcet, *et al.* (2001) *Proc. Natl. Acad. Sci. USA* 98:3416-3421). The invention contemplates use of NTN2L polypeptides and nucleic acids, antigenic fragments thereof, and binding compositions specific for NTN2L polypeptides and nucleic acids, for the treatment and diagnosis of proliferative disorders, e.g., cancer.

III. Myc binding assays.

[0066] Myc targets can be identified by methods sensitive to the binding of Myc to genomic target sequences, such as regulatory sequences containing an E-box. The chromatin

immunoprecipitation (ChIP) method measures binding of Myc to target sequences. This method can involve pre-treating chromatin with formaldehyde to cross-link proteins to DNA; followed by limited fragmentation of chromatin, immunoprecipitation with anti-Myc antibody, with collection of immuno-precipitated genes or gene fragments, followed by their identification or quantitation, e.g., by the PCR method.

[0067] The nucleic acid sequences in non-precipitated and precipitated DNA can be identified by hybridization techniques or by PCR analysis, while the associated proteins can be identified by immunoblotting or amino acid sequencing (Menssen and Hermeking (2002) *supra*; Boyd and Farnham (1999) *Mol. Cell. Biol.* 19:8389-8399; Boyd and Farnham (1997) *Mol. Cell. Biol.* 17:2529-2537; Boyd, *et al.* (1998) *Proc. Natl. Acad. Sci. USA* 95:13887-13892; Frank, *et al.* (2001) *Genes Devel.* 15:2069-2082). Methods using electrophoretic mobility, microarrays, and transcription assays have also been used to identify Myc-targets (Oster, *et al.* (2002) *supra*; Schuhmacher, *et al.* (2001) *Nucl. Acids Res.* 29:397-406; Yu, *et al.* (2002) *J. Biol. Chem.* 277:13059-13066; Coller, *et al.* (2000) *Proc. Natl. Acad. Sci. USA* 97:3260-3265).

IV. Screening for nucleic acids, polypeptides, and binding compositions.

[0068] Cells, tissues, organs, or animals expressing a Myc-regulated gene can be used for screening agents and compositions that modulate gene expression or activity of a polypeptide expressed from a gene of Tables 1 or 2. The cell or animal may comprise or express the natural Myc-regulated gene, or it can be engineered to comprise or express altered levels or muteins. Detection of endogenous and engineered genes in a cell line, cell sample, or tissue sample generally involves detecting changes in levels of the relevant mRNA or polypeptide. Myc-regulated means, e.g., Myc-induced or Myc-suppressed.

[0069] Nucleic acids can be measured by methods dependent on hybridization, such as the TaqMan® technique, see, e.g., Heid, *et al.* (1996) *Genome Res.* 6:989-994; Liu, *et al.* (2002) *Analyt. Biochem.* 300:40-45; Huang, *et al.* (2000) *Cancer Res.* 60:6868-6874; Wittwer, *et al.* (1997) *Biotechniques* 22:130-138; Schmittgen, *et al.* (2000) *Analyt. Biochem.* 285:194-204; Sims, *et al.* (2000) *Analyt. Biochem.* 281:230-232.

[0070] Microarrays can be used for screening, see, e.g., Ausubel, *et al.* (2001) *Curr. Protocols Mol. Biol., Vol. 4*, John Wiley and Sons, New York, NY, pp. 22.0.1-22.3.26; (Huang,

et al. (2000) *Cancer Res.* 60:6868-6874; Ausubel, *et al.* (2001) *Curr. Protocols Mol. Biol., Vol. 4*, John Wiley and Sons, New York, NY, pp. 25.0.1-25B.2.20; Ausubel, *et al.* (2001) *Curr. Protocols Mol. Biol.*, Vol. 3, John Wiley and Sons, New York, NY, pp. 14.0.1-14.14.8; Gray, *et al.* (1998) *Science* 281:533-538; U.S. Pat. No. 6,028,186 issued to Tasset, *et al.*

[0071] Methods for screening and assessing properties of enzymes, e.g., protein kinases are available, see, e.g., Al-Obeidi and Lam (2000) *Oncogene* 19:5690-5701; Ohmi, *et al.* (2000) *J. Biomol. Screen.* 5:463-470; Chapman and Wong (2002) *Bioorganic Medicinal Chem.* 10:551-555; Stratowa, *et al.* (1999) *Anti-Cancer Drug Design* 14:393-402.

[0072] Cells can be screened and purified, e.g., by fluorescent activated cell sorting (FACS), see, e.g., Melamed, *et al.* (1990) *Flow Cytometry and Sorting*, Wiley-Liss, Inc., New York, NY; Shapiro (1988) *Practical Flow Cytometry*, Liss, New York, NY; and Robinson, *et al.* (1993) *Handbook of Flow Cytometry Methods*, Wiley-Liss, New York, NY.

V. Protein purification.

[0073] It is contemplated to purify the polypeptide diagnostics or therapeutics used in the methods of the invention, e.g., antigens, antibodies, and antibody fragments, by methods that are established in the art. Purification can be accomplished by, e.g., immunoprecipitation, ion exchange chromatography, epitope tags, affinity chromatography, and high pressure liquid chromatography, with optional use of detergents, emulsifiers, and stabilizing agents, see, e.g., Dennison and Lovrien (1997) *Protein Expression Purif.* 11:149-161; Murby, *et al.* (1996) *Protein Expression Purif.* 7:129-136; Ausubel, *et al.* (2001) *Curr. Protocols Mol. Biol., Vol. 3*, John Wiley and Sons, New York, NY, pp. 17.0.1-17.23.8; Rajan, *et al.* (1998) *Protein Expression Purif.* 13:67-72; Amersham-Pharmacia (2001) *Catalogue*, Amersham-Pharmacia Biotech, Inc., pp. 543-567, 605-654; Gooding and Regnier (2002) *HPLC of Biological Molecules*, 2nd ed., Marcel Dekker, NY.

VI. Small molecule therapeutics.

[0074] The invention encompasses use of small molecule diagnostics and therapeutics for, e.g., modulating expression and activity of Myc-binding genes or the respective gene products (Tables 1 or 2). Natural products and synthetic compounds are generally known as

"small molecules" when of significantly lesser molecular weight than a typical polypeptide, i.e., significantly lower than 50 kDa. Methods for preparing and using small molecules are described, see, e.g., Al-Obeidi and Lam (2000) *Oncogene* 19:5690-5701; Bishop, *et al.* (2001) *Trends Cell Biol.* 11:167-172; Traxler, *et al.* (2001) *Med. Res. Revs.* 21:499-512; Gray, *et al.* (1998) *Science* 281:533-538; Stratowa, *et al.* (1999) *Anti-Cancer Drug Design* 14:393-402; Rosen (2001) *Cancer J.*, 7 Suppl. 3:S120-128; Sawyers (2002) *Curr. Op. Genetics Devel.* 12:111-115; Rosen (2001) *Cancer J.* 7 Suppl. 3:S120-128; Ripka and Rich (1998) *Curr. Opinion Chemical Biol.* 2:441-452; Hruby, *et al.* (1997) *Curr. Opinion Chemical Biol.* 1:114-119; al-Obeidi, *et al.* (1998) *Mol. Biotechnol.* 9:205-223; Hruby and Balse (2000) *Curr. Med. Chem.* 7:945-970, Martin-Moe, *et al.* (1995) *Pept. Res.* 8:70-76; Guichard, *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:9765-9769; Sloan (1992) *Prodrugs*, Marcel Dekker, New York, NY; Melton and Knox (1999) *Enzyme-Prodrug Strategies for Cancer Therapy*, Plenum Publ. Corp., New York, NY; U.S. Patent No. 6,326,482 issued to Stewart, *et al.* The invention also contemplates the use of pro-drugs, see, e.g., Iyengar, *et al.* (2002) *Cancer Res.* 61:3045:3052; Nishino, *et al.* (1999) *J. Biol. Chem.* 274:32580-32587; Pawlik, *et al.* (2000) *Mol. Ther.* 1:457-463.

VII. Antibodies.

[0075] Antibodies can be raised to a polypeptide gene product, or an antigenic fragment, of a polypeptide of Table 1 or 2, to a chromosomal protein associated with a Myc-binding site or gene of Tables 1 or 2, to biologically or catalytically active or inactive polypeptides, and to native or denatured polypeptides. Anti-idiotypic antibodies are also contemplated.

[0076] Antigenic sequences of the polypeptides corresponding to the genes of Table 2 were determined by a Parker plot using Vector NTI® Suite, Informax, Inc., Bethesda, MD (Parker, *et al.* (1986) *Biochemistry* 18:5425-5431).

[0077] CLCN6 has regions of increased antigenicity at, e.g., amino acids 15-29, 33-42, 46-56, 67-79, 115-124, 232-240, 320-326, 397-412, and 667-694, of AF009247 (Tables 1 and 2).

[0078] CLNS1A has regions of increased antigenicity at, e.g., amino acids at 18-25, 95-108, 137-163, 212-225, of NP_001284 or P54105). NP_001284 or P54105 is the polypeptide of the gene containing the intron of AF148461 (Tables 1 and 2).

[0079] SLC4A2 has regions of increased antigenicity at, e.g., amino acids 91-138, 180-204, 290-323, and 561-578, of U76667 (Tables 1 and 2).

[0080] TAPK has regions of increased antigenicity at, e.g., amino acids 18-28, 114-124, 134-140, 318-326, and 398-413, of AF255613 (Tables 1 and 2).

[0081] Netrin-2-like protein has regions of increased antigenicity at, e.g., amino acids 20-41, 57-78, 225-238, 275-291, and 381-401, of U86758 (Tables 1 and 2).

[0082] Antibodies and binding compositions derived from an antigen-binding site of an antibody are provided. These include human antibodies, humanized antibodies, monoclonal antibodies, polyclonal antibodies, and binding fragments, such as Fab, $F(ab)_2$, and Fv fragments, and engineered versions thereof. The antibody or binding composition can be agonistic or antagonistic. Antibodies that simultaneously bind to a ligand and receptor are contemplated. Monoclonal antibodies will usually bind with a K_D of 1 mM or less, more usually 300 μM or less, typically 100 μM or less, more typically 30 μM or less, preferably at 10 μM or less, and most preferably at 3 μM or less.

[0083] Antibodies can be prepared, see, e.g., Sheperd and Dean (eds.) (2000) Monoclonal Antibodies, Oxford Univ. Press, New York, NY; Kontermann and Dubel (eds.) (2001) *Antibody Engineering*, Springer-Verlag, New York; Harlow and Lane (1988) *Antibodies A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 139-243; Carpenter, *et al.* (2000) *J. Immunol.* 165:6205; He, *et al.* (1998) *J. Immunol.* 160:1029; Tang, *et al.* (1999) *J. Biol. Chem.* 274:27371-27378.

[0084] A humanized antibody contains the amino acid sequences from six complementarity determining regions (CDRs) of the parent mouse antibody, which are grafted on a human antibody framework. An alternative to humanization is to use human antibody libraries displayed on phage or human antibody libraries contained in transgenic animals or cells, see, e.g., Vaughan, *et al.* (1996) *Nat. Biotechnol.* 14:309-314; Barbas (1995) *Nature Med.* 1:837-839; de Haard, *et al.* (1999) *J. Biol. Chem.* 274:18218-18230; McCafferty *et al.* (1990) *Nature* 348:552-554; Clackson *et al.* (1991) *Nature* 352:624-628; Marks *et al.* (1991) *J. Mol. Biol.* 222:581-597; Mendez, *et al.* (1997) *Nature Genet.* 15:146-156; Hoogenboom and Chames (2000) *Immunol. Today* 21:371-377; Barbas, *et al.* (2001) *Phage Display:A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Kay, *et al.* (1996) *Phage*

Display of Peptides and Proteins: A Laboratory Manual, Academic Press, San Diego, CA; de Bruin, *et al.* (1999) *Nat. Biotechnol.* 17:397-399.

[0085] Single chain antibodies, single domain antibodies, and bispecific antibodies are described, see, e.g., Malecki, *et al.* (2002) *Proc. Natl. Acad. Sci. USA* 99:213-218; Conrath, *et al.* (2001) *J. Biol. Chem.* 276:7346-7350; Desmyter, *et al.* (2001) *J. Biol. Chem.* 276:26285-26290, Kostelney, *et al.* (1992) *J. Immunol.* 148:1547-1553; U.S. Pat. Nos. 5,932, 448; 5,532,210; 6,129,914; 6,133,426; 4,946,778.

[0086] Antigen fragments can be joined to other materials, such as fused or covalently joined polypeptides, to be used as immunogens. An antigen and its fragments can be fused or covalently linked to a variety of immunogens, such as keyhole limpet hemocyanin, bovine serum albumin, or ovalbumin (Coligan, *et al.* (1994) *Current Protocols in Immunol., Vol. 2*, 9.3-9.4, John Wiley and Sons, New York, NY). Peptides of suitable antigenicity can be selected from the polypeptide target, using an algorithm, such as those of Parker, *et al.* (1986) *Biochemistry* 25:5425-5432; Jameson and Wolf (1988) *Cabios* 4:181-186; or Hopp and Woods (1983) *Mol. Immunol.* 20:483-489.

[0087] Purification of antigen is not necessary for the generation of antibodies. Immunization can be performed by DNA vector immunization or by immunization with cells bearing the antigen of interest. Immunization with cells may prove superior for antibody generation than immunization with purified antigen, see, e.g., Wang, *et al.* (1997) *Virology* 228:278-284; Meyaard, *et al.* (1997) *Immunity* 7:283-290; Wright, *et al.* (2000) *Immunity* 13:233-242; Preston, *et al.* (1997) *Eur. J. Immunol.* 27:1911-1918; Kaithamana, *et al.* (1999) *J. Immunol.* 163:5157-5164.

[0088] Antibody to antigen binding properties can be measured, e.g., by surface plasmon resonance or enzyme linked immunosorbent assay (ELISA) (Karlsson, *et al.* (1991) *J. Immunol. Methods 145*:229-240; Neri, *et al.* (1997) *Nat. Biotechnol.* 15:1271-1275; Jonsson, *et al.* (1991) *Biotechniques* 11:620-627; Friguet, *et al.* (1985) *J. Immunol. Methods* 77:305-319; Hubble (1997) *Immunol. Today* 18:305-306).

[0089] Antibodies to polypeptides, or to antigenic fragments thereof, expressed from the genes of Tables 1 or 2 but possessing substitutions that do not substantially affect the functional aspects of the nucleic acid or amino acid sequence, are within the definition of the contemplated

invention. Variants with truncations, deletions, additions, and substitutions of regions which do not substantially change the biological functions of these nucleic acids and polypeptides are also within the definition of the contemplated invention.

VIII. Therapeutic compositions.

[0090] The invention provides methods to treat and diagnose various proliferative conditions, e.g., cancer, tumors, metastasis, and angiogenesis.

[0091] Formulations of antibodies, binding composition, polypeptides, antibody mimetics, or small molecule therapeutics, e.g., antisense nucleic acids, are prepared for storage by mixing with physiologically acceptable carriers, excipients, or stabilizers in the form of, e.g., lyophilized powders, slurries, aqueous solutions, see, e.g., Hardman, *et al.* (2001) *Goodman and Gilman's The Pharmacological Basis of Therapeutics,* McGraw-Hill, New York, NY; Gennaro (2000) *Remington: The Science and Practice of Pharmacy*, Lippincott, Williams, and Wilkins, New York, NY; Avis, *et al.* (eds.) (1993) *Pharmaceutical Dosage Forms: Parenteral Medications*, Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) *Pharmaceutical Dosage Forms: Tablets*, Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) *Pharmaceutical Dosage Forms: Disperse Systems*, Marcel Dekker, NY; Weiner and Kotkoskie (2000) *Excipient Toxicity and Safety*, Marcel Dekker, Inc., New York, NY; D'Alessandro (1993) *Cancer Therapy:Differentiation, Immunomodulation and Angiogenesis*, Springer-Verlag, New York, NY; U.S. Pat. Nos. 6,096,728; 6,342,220; and 5,440,021.

[0092] Therapeutic compositions comprising an antibody or small molecule can be administered, e.g., by systemic, intraperitoneal, intramuscular, dermal, subcutaneous, oral, nasal, pulmonary, suppository, and intratumor routes. Sustained-release preparations, liposomes, aerosols, or viral vectors can supply the therapeutic composition by the contemplated method, see, e.g., Sidman *et al.* (1983) *Biopolymers*, 22:547-556; Langer *et al.* (1981) *J. Biomed. Mater. Res.* 15:167-277; Langer (1982) *Chem. Tech.* 12:98-105; Lasic and Papahadjopoulos (eds.) (1998) *Medical Applications of Liposomes*, Elsevier Health Sciences, Phila., PA; Janoff (ed.) (1999) *Liposomes: Rational Design*, Marcel Dekker, Inc., NY, NY; Knowles, *et al.* (1995) *New Engl. J. Med.* 333:823-831; U.S. Pat. Nos. 6,387,404 and 6,375,972.

[0093] An "effective amount" of antibody or other therapeutic, or diagnostic, to be employed will depend, i.e., upon the objectives, the route of administration, the type of antibody employed, and the condition of the patient or subject. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. Typically, the clinician will administer the antibody until a dosage is reached that achieves the desired effect. The progress of this therapy is easily monitored by conventional assays. An effective amount of therapeutic will decrease the symptoms typically by at least about 10%; usually by at least 20%; preferably at least about 30%; more preferably at least about 50%; and most preferably at least about 90%. Guidance in therapeutic and diagnostic methodology is available, see, e.g., Maynard, *et al.* (1996) *A Handbook of SOPs for Good Clinical Practice*, Interpharm Press, Boca Raton, FL; Dent (2001) *Good Laboratory and Good Clinical Practice*, Urch Publ., London, UK.

[0094] As a general proposition, the initial pharmaceutically effective amount of the antibody administered parenterally will be in the range of about 0.1 μg/kg to 10 mg/kg of the patient's body weight per day, ordinarily 0.1 μg/kg/day to 1.0 mg/kg/day, preferably 0.1 μg/kg/day to 0.1 mg/kg/day, more preferably 0.1 μg/kg/day to 0.01 mg/kg/day, and most preferably 0.1 μg/kg/day, or less. The desired dosage can be delivered by a single bolus administration, by multiple bolus administrations, or by continuous infusion administration of antibody, depending on the pattern of pharmacokinetics that the practitioner wishes to achieve. These suggested amounts of antibody are subject to a fair amount of therapeutic discretion. The key factor in selecting an appropriate dose and scheduling is the result obtained.

[0095] In the treatment and prevention of an inflammatory disorder the therapeutic composition will be formulated, dosed, and administered in a fashion consistent with good medical practice. The "therapeutically effective amount" of antibody or binding composition to be administered will be the minimum amount necessary to prevent, ameliorate, or treat the inflammatory or proliferative disorder while minimizing possible toxic effects to the host or patient.

IX. Kits.

[0096] The invention provides methods of using the Myc-binding genes of Tables 1 or 2, expressed nucleic acids, expressed polypeptides, and binding compositions thereto, in a diagnostic kit. Also encompassed is use of antigenic fragments, muteins, metabolites, and chemical and metabolic breakdown products of the polypeptides of Tables 1 or 2. Typically, the kit will have a compartment containing a polypeptide of Tables 1 or 2, or an antigenic fragment thereof, a binding composition, or a nucleic acid, e.g., a nucleic acid probe or primer.

[0097] The kit can comprise, e.g., a reagent and a compartment, a reagent and instructions for use, or a reagent with both a compartment and instructions for use. The reagent can comprise a polypeptide of Tables 1 or 2, or an antigenic fragment thereof, a binding composition, or a gene or nucleic acid of Tables 1 or 2. A kit for determining the binding of a test compound or test binding composition to a target can comprise a control compound, a labeled compound, and a method for separating free labeled compound from bound labeled compound. Conjugated antibodies are useful for diagnostic or kit purposes, and include, e.g., antibodies coupled to dyes, isotopes, enzymes, and metals, see, e.g., Le Doussal, *et al.* (1991) *J. Immunol.* 146:169-175; Gibellini, *et al.* (1998) *J. Immunol.* 160:3891-3898; Hsing and Bishop (1999) *J. Immunol.* 162:2804-2811; Everts, *et al.* (2002) *J. Immunol.* 168:883-889. Diagnostic assays can be used with biological matrices such as live cells, cell extracts or cell lysates, fixed cells, cell cultures, bodily fluids, or forensic samples. Various assay formats are available, e.g., radioimmunoassays (RIA), ELISA, and lab on a chip (U.S. Pat. Nos. 6,176,962 and 6,517,234). Numerous methods are available for separating bound ligand from free ligand, or bound test compound from free test compound, e.g., use of ligands or test compound immobilized by adhesion to plastic, and couplings involving a complex of antigen and antibody, biotin and avidin, and biotin and streptavidin.

X. Uses.

[0098] The present invention provides methods and reagents that will find use in therapeutic and diagnostic applications, e.g., for the treatment and diagnosis of cancer and other proliferative conditions. A reagent sensitive to a single Myc-binding gene or gene product, or to a group of Myc-binding genes or gene products of Tables 1 or 2, is expected to be useful as a

probe in antibody-based assays, FACS assays, histological assays, nucleic acid hybridization-based assays, PCR-based assays, and the like.

[0099] The invention provides a binding composition specific for at least one ion transporter, e.g., CLCN6, CLN1SA, and SLC4A2, specific for at least one protein kinase, e.g., TAPK; or specific for at least one agent that guides cell growth, guides the direction of cell growth, or modulates apoptosis, e.g., NTN2L (Tables 1 and 2).

[0100] The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the inventions to the specific embodiments.

EXAMPLES

I. General Methods.

[0101] Some of the standard methods are described or referenced, see e.g., Maniatis, *et al.* (1982) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor Press; Sambrook and Russell (2001) *Molecular Cloning, 3rd ed.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Wu (1993) *Recombinant DNA, Vol. 217*, Academic Press, San Diego, CA; Innis, *et al.* (eds.) (1990) *PCR Protocols: A Guide to Methods and Applications*, Academic Press, N.Y. Standard methods are also found in Ausbel, *et al.* (2001) *Current Protocols in Molecular Biology, Vols.1-4*, John Wiley and Sons, Inc. New York, NY, which describes cloning in bacterial cells and DNA mutagenesis (Vol. 1), cloning in mammalian cells and yeast (Vol. 2), glycoconjugates and protein expression (Vol. 3), and bioinformatics (Vol. 4).

[0102] Methods for protein purification such as immunoprecipitation, column chromatography, electrophoresis, isoelectric focusing, centrifugation, and crystallization, are described (Coligan, *et al.* (2000) *Current Protocols in Protein Science, Vol. 1*, John Wiley and Sons, Inc., New York). Chemical analysis, chemical modification, post-translational modification, and glycosylation of proteins is described (Coligan, *et al.* (2000) *Current Protocols in Protein Science, Vol. 2*, John Wiley and Sons, Inc., New York). The production, purification, and fragmentation of polyclonal and monoclonal antibodies is described (Coligan, *et al.* (2001) *Current Protcols in Immunology, Vol. 1*, John Wiley and Sons, Inc., New York; Harlow and Lane (1999) *Using Antibodies*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY); Harlow and Lane (1988) *supra*).

[0103] Cell culture techniques are described in Doyle, *et al.* (eds.) (1994) *Cell and Tissue Culture: Laboratory Procedures*, John Wiley and Sons, NY. FACS analysis is described in Melamed, *et al.* (1990) *Flow Cytometry and Sorting*, Wiley-Liss, Inc., New York, NY; Shapiro (1988) *Practical Flow Cytometry*, Liss, New York, NY; and Robinson, *et al.* (1993) *Handbook of Flow Cytometry Methods*, Wiley-Liss, New York, NY.

[0104] Methods for the diagnosis and treatment of cancer and other proliferative disorders, angiogenesis, and apoptosis, are described (Warrington, *et al.* (2002) *Microarrays and*

Cancer Research, Eaton Pub. Co. Natick, MA; Fletcher (2000) *Diagnostic Histopathology of Tumors*, Churchill Livingstone, St. Louis, MO; Chabner (2001) *Cancer Chemotherapy and Biotherapy, 3rd ed.*, Lippincott Williams and Wilkins, Phila., PA; Casciato and Lowitz (2000) *Manual of Clinical Oncology, 4th ed.*, Lippincott, Williams, and Wilkins, Phila., PA; Rubanyi (2000) *Angiogenesis in Health and Disease*, Marcel Dekker, New York, NY; Cotter (1997) *Techniques in Apoptosis*, Univ. of Calif. Press, Berkeley, CA; Leblanc (2002) *Apoptosis:Techniques and Protocols*, Humana Press, Totawa, NJ; Hughes and Mehmet (2002) *Cell Proliferation and Apoptosis*, Springer Verlag, New York, NY.

II. Gene fragments producing a positive signal in the ChIP assay.

[0105] 2224 E-boxes were screened by the ChIP assay. The E-boxes and associated human genes that screened positive are shown (Table 1). The structure of the E-box associated with each gene is shown in the Abstract of Mol, *et al.* (1995) *Mol. Cell. Biol.* 15:6999-7009. U937 cells, HL60 cells, P496-3 cells (-Tet), P496-3 cells (+Tet), T98G cells, and WS1 cells served as the source of chromatin in the ChIP assays. A separate group of E-boxes, randomly chosen from chromosome 21, was subjected to the ChIP test, using anti-Myc as the test antibody. The results from these randomly chosen samples served as a control.

[0106] Following immunoprecipitation and quantitation of the immunoprecipitated gene by the PCR technique, the following calculations were made: (1) Comparison of PCR signal from chromatin collected with anti-Myc antibody (experimental) with PCR signal from unfractionated chromatin, expressed as a percentage; (2) Comparison of PCR signal from chromatin subjected to a control immunoprecipitation (control) with PCR signal from unfractionated chromatin, expressed as a percentage; (3) Comparison of (1) with (2) to provide an apparent value for "fold enrichment."

[0107] Two criteria must be satisfied for a gene/E box to be considered to screen positive in the ChIP assay. First, the PCR signal from the + anti-Myc antibody ChIP test (see (1) above) must be 0.1% or greater than that of the PCR signal from the unfractionated, sonicated chromatin, for a given gene target. Second, comparison between (1) and (2) (see above) must be 10-fold or greater. In other words, a site was classed as positive if it was enriched by at least 0.1% in Myc immunoprecipitates and was enriched by at least 10-fold over its value in control

precipitates. A gene satisfying both criteria was considered to give a positive result by the ChIP assay. Positively screening genes/E boxes are listed in Table 1.

[0108] Alternate definitions for a positive-screening ChIP result may be devised, e.g, those that are more or less stringent than the above definition.

[0109] All of the genes of Table 1 show a "fold-enrichment value" of 10-fold or greater and gave a PCR signal from the + anti-Myc antibody ChIP test (once recovered in the immunoprecipitate) that was 0.1% or greater than that of the PCR signal from the unfractionated, sonicated chromatin (Table 1). Negative controls in the ChIP assays include genes containing no E-boxes, e.g., PCNA, acetylcholine receptor, and topoisomerase II, and genes containing an E-box but where Myc did not bind, e.g., glucokinase; glycine methyltransferase; socs-2 (Frank, *et al.* (2001) *supra*).

Table 1.

Accession Number	**Gene Definition**	**Position of E-box**
AB004270_p1	hMCM7 gene promoter region.	-74
AB004270_p1	hMCM7 gene promoter region.	-74
AB009271_p1	BCNT, partial cds.	915
AB009589_i1	Osteomodulin	5231
AB009666_p1	Klotho gene, exon 1.	943
AB012942_i1	AOC2, retina-specific amine oxidase, exon 1	3353
AB012943_p1	AOC2 gene for retina-specific amine oxidase, exons 2a, 2b, 3 and 4 and complete cds	1038
AB013139_p1	NBS1, complete cds.	1001
AB014460_p1	TSC2 and NTHL1 head-to-head genes	-1368
AB014460_p1	TSC2 and NTHL1 head-to-head genes	-9
AB014460_p1	TSC2 and NTHL1 head-to-head genes	289
AB015961_i1	IL-18, intron 1 and exon 2.	-3195
AB015961_i1	IL-18, intron 1 and exon 2.	-2819
AB016194_p1	Elk1 oncogene, complete cds.	-4
AB016194_p1	Elk1 oncogene, complete cds.	1599

AB016243_p1	Regulatory factor 2 of sodium/hydrogen exchanger isoform A3, complete cds	657
AB016243_p1	Regulatory factor 2 of sodium/hydrogen exchanger isoform A3, complete cds	820
AB017018_p1	JKTBP2, JKTBP1, complete cds.	-1147
AB017018_p1	JKTBP2, JKTBP1, complete cds.	-939
AB017710_p1	U50HG genes for U50' snoRNA and U50 snoRNA, complete sequence.	-562
AB017710_p1	U50HG genes for U50' snoRNA and U50 snoRNA, complete sequence	161
AB019198_i1	Caspase-9, exon 2.	-98
AB019581	wee1	166
AB019581	wee1	-38
AB019581	wee1	-832
AB020236	RPL27a	424
AB028893_p2	RPL13A, U32, U33, U34, U35, RPS11, U35 genes for ribosomal protein L13a and S11, U32, U33, U34, U35, and U35 snoRNA complete cds and sequence	490
AB028893_p2	RPL13A, U32, U33, U34, U35, RPS11, U35 genes for ribosomal protein L13a and S11, U32, U33, U34, U35, and U35 snoRNA complete cds and sequence	-220
AB030817_p1	Hper1 gene for period1, complete cds.	-1360
AB030817_p1	Hper1 gene for period1, complete cds.	-629
AB032481_p1	HOXD13 gene for homeobox transcription factor, complete cds	691
AB038980, NT_005229	caspase 8	202
AB038980, NT_005229	caspase 8	558
AF210257, NT_005229	caspase 8	24829
AF210257, NT_005229	caspase 8	31045
AF210257, NT_005229	caspase 8	32634
AB046408	RPL22	74
AC007114	AKAP1	-1994

AC007114	AKAP1	-15
AC007114	AKAP1	900
AC008532	PUMA/JFY1/BBC3	773
AC008532	PUMA/JFY1/BBC3	1628
AF332559	PUMA/JFY1/BBC3	-379
AF411827	PUMA/JFY1/BBC3	-1705
AF001689	RPL23a	2055
AF001976	Type I sigma receptor	396
AF005891	Na K-ATPase beta-3 subunit (atp1b3), exon 2.	intron 1 (3'end)
AF009247_p1	Putative chloride channel gene (CLCN6), partial promoter and exon 1	-91
AF009247_p1	Putative chloride channel gene (CLCN6), partial promoter and exon 1	-68
AF009247_p1	Putative chloride channel gene (CLCN6), partial promoter and exon 1	244
AF010238_p1	von Hippel-Lindau tumor suppressor (VHL) gene	911
AF010317_p1	Pig3 (PIG3) gene, partial cds.	1269
AF015947	cad gene, promoter region.	-2025
AF015947_p1	cad gene, promoter region.	-454
AF015947_p1	cad gene, promoter region.	-483
AF018631	Biotinidase (BTD), exons 2-4	-364
AF025878_p1	Inosine monophosphatase 2 (IMPA2) gene, promoter sequence	-690
AF026855	Mitochondrial short-chain L-3-hydroxyacyl-CoA dehydrogenase (HADHSC)	intron 1
AF029081	14-3-3 sigma protein promoter and gene	-8169
AF029081	14-3-3 sigma protein promoter and gene	-7215
AF033033_p1	TIRC7 protein (TCIRG1) gene, complete cds.	603
AF035427_p1	Bone morphogenetic protein 4 (BMP4) gene, promoter 1 and exon 1	-196
AF035753_p1	Endoglin (END) gene, promoter region, exon 1 and partial cds	-1001
AF035753_p1	Endoglin (END) gene, promoter region, exon 1 and partial cds	338
AF039954_p1	CC chemokine LCC-1 precursor, gene, complete cds.	-147

AF042836	Cystathionine beta-synthase (CBS) gene, major and minor alternative splice products, complete cds	-1588
AF042836	Cystathionine beta-synthase (CBS) gene, major and minor alternative splice products, complete cds	-573
AF042836	Cystathionine beta-synthase (CBS) gene, major and minor alternative splice products, complete cds	737
AF042836	Cystathionine beta-synthase (CBS) gene, major and minor alternative splice products, complete cds	1434
AF042848_p1	EMMPRIN gene, promoter and exon 1.	-228
AF046925_p1	Hepatocyte growth factor receptor (c-met) gene, promoter sequence	-385
AF049198_i1	Sodium iodide symporter gene, intron 1, 5' partial	intron 1
AF050068_p1	Growth arrest specific 11 GAS11) gene, exon 1.	-208
AF050115_p1	Hypoxia-inducible factor 1 alpha subunit (HIF1A) gene, exon 1	-276
AF050115_p1	Hypoxia-inducible factor 1 alpha subunit (HIF1A) gene, exon 1	-276
AF055992_p1	Duffy antigen/chemokine receptor (FY) gene, FY*X allele, complete cds	-568
AF059650_p1	Histone deacetylase 3 (HDAC3) gene, complete cds.	-1525
AF060494_p1	Ubiquitin binding protein p62 gene, promoter and partial cds	-1344
AF065396_p1	Retinoic X receptor B gene, complete cds.	796
AF067130_p1	Protein phosphatase-1 regulatory subunit 7 (PPP1R7) gene, exon 1	469
AF067572_p1	Signal transducer and activator of transcription 6 (STAT6) gene, exons 1 through 12	-1754
AF067572_p1	Signal transducer and activator of transcription 6 (STAT6) gene, exons 1 through 12	-823
AF067572_p1	Signal transducer and activator of transcription 6 (STAT6) gene, exons 1 through 12	-148
AF067844_p1	Chromosome 10 clone PTEN, complete sequence.	-1374
AF067844_p1	Chromosome 10 clone PTEN, complete sequence.	-1148
AF069984_p1	Nitrilase homolog 1 (NIT1) gene, alternatively spliced product, complete cds	-621
AF069984_p1	Nitrilase homolog 1 (NIT1) gene, alternatively spliced product, complete cds	1459
AF071540_p1	Erythrocyte membrane protein 4.2 gene, promoter sequence	-316
AF071541	Erythrocyte membrane protein 4.2 gene, intron 1	intron 1

AF072562_p1	Zinc finger protein 74 (ZNF74) gene, exons 2a and 2b.	-40
AF074333_p1	Glycogen synthase kinase 3 beta gene, promoter region and partial cds	-323
AF076613	Promyelocytic leukemia zinc finger (PLZF)	1299
AF078694_p1	*Alpha 6 integrin subunit (ITGA6) gene, promoter sequence*	-423
AF086788_p1	Aconitase (ACO2) gene, nuclear gene encoding mitochondrial protein, exon 1	-141
AF086926_p1	Dynactin 1 (DCTN1) gene, exon 1.	-61
AF088888_p1	Retinoic acid receptor alpha (RARA) gene, exon 1.	34
AF092906_p1	*Ribosomal protein S19 (RPS19) gene, exon 1 and 2.*	-359
AF092926_p2	Microsomal glutathione transferase (MGST1) gene, exons 1 and 2, alternatively spliced products	384
AF104233_p1	ADP-ribosylation factor 4 (ARF4) gene, exon 1.	-670
AF112181_p1	Interferon regulatory factor 3 (IRF3) gene, promoter region and partial cds	517
AF112229_p1	CD30 protein (CD30) gene, promoter, exon 1, and partial cds	229
AF112229_p1	CD30 protein (CD30) gene, promoter, exon 1, and partial cds	23
AF112482_p1	Aldehyde reductase (AKR1A1) gene, exon 1 and promoter sequence	-1622
AF126958_p1	Outer membrane receptor Tom20 (TOM20) gene, exon 1; nuclear gene encoding mitochondrial protein	-188
AF128893_p1	telomerase reverse transcriptase (TERT)	-3929
AF128893_p1	telomerase reverse transcriptase (TERT)	-179
AF128893_p1	Telomerase reverse transcriptase (TERT)	29
AF128893_p1	Telomerase reverse transcriptase (TERT)	1842
AF132894_p1	Cathepsin F (CTSF) gene, complete cds.	-371
AF132894_p1	Cathepsin F (CTSF) gene, complete cds.	-152
AF132894_p1	Cathepsin F (CTSF) gene, complete cds.	-80
AF134201_p1	Prostaglandin E2 receptor EP2 subtype (PTGER2) gene, exon 1	1313
AF135372_p1	Synaptobrevin 2 (VAMP2) gene, complete cds.	-821
AF142779_p1	BN51 protein gene, promoter region and partial cds.	233
AF144014	mdm2	175
AF145047_p1	Heme oxygenase-1 gene, promoter region; and exon 1, partial sequence	-43

AF147742_p1	Myeloid cell differentiation protein (MCL1) gene, promoter and complete cds	-587
AF148461_i1	CLNS1A	intron 1
AF148461	CLNS1A	intron 1
AF149773_p1	NOD1 protein (NOD1) gene, exons 1, 2, and 3.	-1287
AF149773_p1	NOD1 protein (NOD1) gene, exons 1, 2, and 3.	-489
AF156731_p1	Smad7 gene, promoter region.	-91
AF163763_p1	Elongation factor 1 A-2 (EF1A-2) gene, complete cds.	-528
AF163763_p1	Elongation factor 1 A-2 (EF1A-2) gene, complete cds.	1411
AF163776_p1	TCF1 gene, partial cds.	-1004
AF166335_p1	Integrin alpha 6 (ITGA6) gene, exon 1.	-407
AF175325_p1	Eukaryotic initiation factor 4AI (EIF4A1) gene, partial cds	-295
AF175325_p1	Eukaryotic initiation factor 4AI (EIF4A1) gene, partial cds	1125
AF187320	Transferrin receptor	559
AF196969_p1	Phenylalkylamine binding protein gene, complete cds; MG81 protein gene, partial cds; putative RNA-binding protein 3 RNP gene, complete cds; and MG21 pseudogene, complete sequence	333
AF198614_p1	Mcl-1 (MCL-1) and Mcl-1 delta S/TM (MCL-1) genes, alternative spliced forms, complete cds	-579
AF207550_p1	Protein translocase, JM26 protein, UDP-galactose translocator, pim-2 protooncogene homolog pim-2h, and shal-typ potassium channel genes, complete cds; JM12 protein an transcription factor IGHM enhancer 3 genes, partial cds.	-1587
AF208234_p1	Cystatin B (CSTB) gene, promoter region and complete cds	11
AF208501_p1	Uncoupling protein 3 (UCP3) gene, promoter and exon 1.	-1050
AF209746_p1	Beta tropomyosin (TPM2) gene, exons 1 through 8 and partial cds	-762
AF223404	WNT1 inducible signaling pathway protein 1 (WISP1) gene, promoter and partial cds	-4602
AF223404_p1	WNT1 inducible signaling pathway protein 1 (WISP1) gene, promoter and partial cds	-1101
AF224272_p1	Cyclin dependent kinase 4, promoter region.	-125
AF224272_p1	Cyclin dependent kinase 4, promoter region.	-198
U37022_p1, AF224272_p1	cdk4	-29

U37022_p1, AF224272_p1	cdk4	455
AF239710_p1	DNA polymerase delta small subunit (POLD2) gene, exons 1 through 11 and complete cds	287
AF255613_p1	Teratoma-associated tyrosine kinase (TAPK) gene, exons 1 through 6 and partial cds	349
AF257772	RNA binding protein MCG10 gene	-2257
AF258623_p1	ATP binding cassette transporter 1 (ABCA1) gene, promoter and exon 1	-64
AF258674_p1	MUCDHL (MUCDHL) gene, complete cds, alternatively spliced	-469
AF270493	Id2	-1590
AF289220	BCL2L12	65
AF293386_p1	Eukaryotic translation initiation factor 5AII (EIF5A2) gene, exons 1, 2, and 3	-186
AJ001686_p1	NKG2F gene.	-1322
AJ002311_i1	Synaptogyrin 2	intron 1
AJ006239_	QDPR; dihydropteridine reductase	intron 1
AJ009866_p1	pex3 gene (joined CDS, promoter and exon 1).	-1086
AJ009866_p1	pex3 gene (joined CDS, promoter and exon 1).	-752
AJ010341_p1	PISSLRE gene, exons 1, 2, and 3 and joined CDS.	779
AJ010395_i1	DKC1	680
AJ010395_i1	DKC1	653
AJ011802_p1	OZF gene exon 1.	-676
AJ011802_p1	OZF gene exon 1.	27
AJ012453_p1	MUC5B gene proximal 5' flanking region.	1027
AJ131016_p1	SCL gene locus.	-147
AJ131757	olr1	2481
AJ131612	dic	1749
AJ224639_p1	Surf-5 and Surf-6 genes.	-368
AJ224639_p1	Surf-5 and Surf-6 genes.	-219
aj238481_p1	FBP2 gene fructose-1,6-bisphosphatase 2	-1450
AJ238482_i1	FBP2; fructose-1,6-bisphosphatase 2	1291

AJ238511_i1	MVP	intron 1
aj238592	SLAP (src-like adaptor protein)	-2974
AJ243297_i1	RET	-837
AJ245489_p1	GCGR gene for glucagon receptor, promoter I.	-1100
AJ249162	Enhancer from ISG20	
AJ249275_p2	Partial MTHFR gene for methylenetetrahydrofolate reductase	-1108
AJ249275_p2	Partial MTHFR gene for methylenetetrahydrofolate reductase	-797
AJ249275_p2	Partial MTHFR gene for methylenetetrahydrofolate reductase	-774
AJ250235_i1	FECH gene for ferrochelatase	5944
AJ250249_p1	Partial Mif1/KIAA0025 gene, 5'-upstream region.	-475
AJ250249_p1	Partial Mif1/KIAA0025 gene, 5'-upstream region.	-168
AJ250915_p1	p10 gene for chaperonin 10 (Hsp10 protein) and p60 gene for chaperonin 60 (Hsp60 protein)	232
AJ250915_p1	p10 gene for chaperonin 10 (Hsp10 protein) and p60 gene for chaperonin 60 (Hsp60 protein)	-522
AJ272029_p1	Partial CD30 gene for cytokine receptor CD30 and promoter region	951
AL021154	Id3	-2895
AL022312_p2	ATF4 gene	-86
AL121928_p1	DNA sequence from clone RP11-18I14, gene bA18I14.4	-1514
AL133551_p1	SIRT1 gene (Sir2-like proteins (siruitins) type 1)	-1049
AL133551_p1	SIRT1 gene (Sir2-like proteins (siruitins) type 1)	-423
D00591_p1	RCC1 gene, exons 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, complete cds	1055
D13156_p1	Elafin, complete cds.	343
D13370_p1	APX gene encoding APEX nuclease, complete cds.	-9
D14668_p1	Proteasome HC5, 5'-flanking region.	47
D17616_p1	N-acetylgalactosamine 6-sulfate sulfatase (GALNS), exon 1	-187
D21801_p1	Proteasome subunit HC8, 5'-flanking region.	-660
D28877_i1	hnRNP protein A2/B1	2218
D38592_i1	MTH1 gene for 8-oxo-dGTPase	-56
D50030_p1	Hepatocyte growth factor activator, complete cds	1873

D63395_p1	NOTCH4, partial cds.	870
D63861_p1	Cyclophilin 40, complete cds.	499
D63861_p1	Cyclophilin 40, complete cds.	949
D85429_p1	Heat shock protein 40, complete cds.	-1346
D85922_i1	single-minded 2	177
D85922_i1	single-minded 2	-360
D87675_p1	Amyloid precursor protein, complete cds.	3267
D87675_p1	Amyloid precursor protein, complete cds.	568
D87943_i1	Alpha(1,2)fucosyltransferase	intron 1
D90084_p1	Pyruvate dehydrogenase(EC 1.2.4.1)alpha subunit gene,exons 1-11	826
J00153_p1	Alpha globin psi-alpha-1, alpha-2 and alpha-1 genes, complete cds	371
J03466_p1	Insulin receptor gene, exon 1, clone p-lambda EA2.	-876
J03764_p1	Plasminogen activator inhibitor-1 gene, exons 2 to 9.	-556
J03764_p1	Plasminogen activator inhibitor-1 gene, exons 2 to 9.	-440
J04038_p1	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene, complete cds	666
J04111_p1	c-jun proto oncogene (JUN), complete cds, clone hCJ-1.	946
J04201_p1	Beta-polymerase gene, exons 1 and 2.	-525
J04809_p1	Cytosolic adenylate kinase (AK1) gene, complete cds.	-818
J04988_p1	90 kD heat shock protein gene, complete cds.	374
J05096_p1	Na,K-ATPase subunit alpha 2 (ATP1A2) gene, complete cds.	1228
J05253_i1	Interstitial retinol-binding prot. prec. (IRBP)	4328
K02402_i1	Coagulation factor IX	2866
K03014_p1	MHC class II HLA-SB-beta-1 gene (untyped), clone LC11.	1564
K03021_p1	Tissue plasminogen activator (PLAT) gene, complete cds.	557
L04147_p1	Neurofilament light chain (NEFL) gene, promoter region.	-1239
L06162_p1	Breast cancer-associated antigen (DF3) gene, 5' end and promoter region	-1583
L06484_p1	Acetylcholinesterase (ACHE) gene, exons 1-2, and promoter region	-262
L07485_p1	Deoxycytidine kinase gene, promoter region.	-450

L10137_p1	Histone (H2AZ) gene, promoter sequence.	-392
L10347_i1	Pro-alpha1 type II collagen (COL2A1)	1219
L10822_p1	Gastrin receptor gene, complete cds.	1872
L11016_p1	Lymphotoxin-beta gene, complete cds.	-1531
L12399_p1	Nuclear lamin A and nuclear lamin C gene, exon 1.	-707
L13391_p1	Helix-loop-helix basic phosphoprotein (G0S8) gene, complete cds	-823
L14272_p1	Prohibitin (PHB) gene, exons 1-7.	-352
L16842_p1	Ubiquinol cytochrome-c reductase core I protein mRNA, complete cds	883
L19314	HRY (Hairy, or HHL, HES-1)	-1260
L21905_p1	Troponin I, slow-twitch isoform (TNNI1) gene, exon 1.	-567
L22298_p1	Moesin gene.	-335
L24442_p1	Interferon regulatory factor 2 (IRF2) gene, 5' flank.	-403
L25932_p1	Integral nuclear envelope inner membrane protein (LBR) gene, 5' UTR, exons 1 and 2	1019
l27148	Galanin gene, 5' end.	-2487
L27148_p1	Galanin gene, 5' end.	-1383
L27587_i1	CD79b/Ig beta/B29	-644
L29530_i1	CACNL1A1 calcium channel L-type alpha 1 sub.	-129
L29766_i1	Epoxide hydrolase (EPHX1)	-2207
X77738_p1	Red cell anion exchanger (EPB3, AE1, Band 3) gene, 3' region	-940
L39891_i1	Polycystic kidney disease-associated protein (PKD1)	10910
L41560_i1	PCBD; pterin-4a-carbinolamine dehydratase	-4219
L41919_p1	Candidate tumor suppressor HIC-1 (HIC-1) gene, complete cds	1338
L44140_p1	Chromosome X region from filamin (FLN) gene to glucose-6-phosphate dehydrogenase (G6PD) gene, complete cds's	1176
L44140_p3	Chromosome X region from filamin (FLN) gene to glucose-6-phosphate dehydrogenase (G6PD) gene, complete cds's	470
M10090_p1	Myoglobin gene, exon 1.	244
M11166_p1	Prepro-8-arginine-vasopressin-neurophysin II gene, complete cds	-152
M11726_p1	Pancreatic polypeptide gene, complete cds.	235

M12036_i1	HER2	625
M13934	RPS14	4100
M19283_p1	Cytoskeletal gamma-actin gene, complete cds.	-142
M19508_p1	Myeloperoxidase gene, exons 1-4.	1029
M19720_p1	L-myc protein gene, complete cds.	1238
M20317_p1	Alpha-galactosidase A gene, exon 1.	-221
M20444_p1	Nucleus-encoded mitochondrial aldehyde dehydrogenase 2 (ALDH2) gene, exon 1	-123
M25161_p1	Na,K-ATPase beta subunit (ATP1B) gene, exons 1 and 2.	-1119
M26167_p1	Platelet factor 4 varation 1 (PF4var1) gene, complete cds.	314
M27274_p1	Prostate specific antigen gene, complete cds.	-316
M29186_p1	Triglyceride lipase gene, exon 1.	-765
M29186_p1	Triglyceride lipase gene, exon 1.	-279
M30137_p1	ets2 protein gene, 5' flank.	-398
M31303_p1	Oncoprotein 18 (Op18) gene, complete cds.	-39
M31303_p1	Oncoprotein 18 (Op18) gene, complete cds.	-870
M31303_p1	Oncoprotein 18 (Op18) gene, complete cds.	-39
M31731_p1	Chronic lymphatic leukemia protein (bcl-3) gene, exon 1, clone cLK2	-731
M32405_p1	Homologue of rat insulinoma gene (rig), exons 1-4.	768
M33132_p1	Proliferating cell nucleolar protein P120 gene, exons 1-15.	-336
M35425_p1	Hepatic lipase gene, exon 1.	-311
M35425_p1	Hepatic lipase gene, exon 1.	-798
M37065_p1	Glutathione S-transferase (GST-pi) pi gene, 5'-flanking region	-1820
M58602_p1	Platelet-derived endothelial cell growth factor gene, exons 1 through 10	-442
M58602_p1	Platelet-derived endothelial cell growth factor gene, exons 1 through 10	232
M60436_p1	Poly(ADP-ribose) polymerase gene, 5' end.	106
m60556	Transforming growth factor beta-3	-2160
M60556_p1	Transforming growth factor beta-3 gene, 5' end.	-1046
M60858_p1	Nucleolin gene, complete cds.	406

M60858_p1	Nucleolin gene, complete cds.	429
M61170_p1	Polymorphic epithelial mucin (PEM) gene, complete cds.	-1510
M61170_p1	Polymorphic epithelial mucin (PEM) gene, complete cds.	-1595
M63544	SLBP (histone stem-loop binding protein)	
M64231_p1	Spermidine synthase gene, complete cds.	549
M64231_p1	Spermidine synthase gene, complete cds.	-191
M64280_p1	Complement receptor 2 (CR2, CD21) gene, promoter region.	-130
M65001_p1	Intercellular adhesion molecule 1 (ICAM-1) gene, exon 1.	274
M68882_p1	Steroid 5-alpha-reductase gene, exon 1.	-497
M74239_p1	(dx patient) phosphoprotein (p18) gene, exon 1.	-39
M77232_p1	Ribosomal protein S6 gene, complete cds and flanking regions.	461
M81834_p1	Alpha-2 collagen type VI gene, exons 1 and 2.	-1240
M84327_p1	ADP-ribosylation factor 1 gene, exon 1.	-447
M86181_p1	Prosaposin (PSAP) gene.	-1447
M87841_p1	Histone (H10) gene, 5' region.	-1334
M87843_p1	Transforming growth factor beta-2 gene, 5' end.	-49
M87843_p1	Transforming growth factor beta-2 gene, 5' end.	752
M88003_p1	S-adenosylmethionine decarboxylase (AMD1) gene, exon 1.	-399
M90058_p1	Serglycin gene, exons 1,2, and 3.	-1703
M91463_p1	Glucose transporter (GLUT4) gene, complete cds.	1038
M92444_p1	Apurinic/apyrimidinic endonuclease (HAP1) gene, complete cds	-33
M94363_p1	Lamin B2 (LAMB2) gene and ppv1 gene sequence.	421
M94363_p1	Lamin B2 (LAMB2) gene and ppv1 gene sequence.	1434
M94579_p1	Carboxyl ester lipase (CEL) gene, complete cds.	-353
M95623_p1	Hydroxymethylbilane synthase gene, complete cds.	-453
M95623_p1	Hydroxymethylbilane synthase gene, complete cds.	-62
M96264_p1	Galactose-1-phosphate uridyl transferase (GALT) gene, complete cds	-89
M97911_p1	Wegener's granulomatosis autoantigen proteinase 3 gene, exons 1, 2, 3, 4, and 5	72
M97911_p1	Wegener's granulomatosis autoantigen proteinase 3 gene, exons 1, 2, 3, 4, and 5	1699

M99703_p1	Class II AP endonuclease (APE) gene, partial CDS.	-395
NT_030106	fra-1	913
NT_030106	fra-1	913
S56449_p1	(PTMA) prothymosin alpha {5' region, promoter} [human, Genomic, 2025 nt]	-1170
S74230_p1	E2F1	-1366
S74230_p1	E2F1	-364
U00239_p1	GPAT and AIRC genes promoter sequence.	-189
U01317_p1	Beta globin region on chromosome 11.	-1295
U02509_p1	Adenomatous polyposis coli (APC) gene, promoter sequence.	-48
U02509_p1	Adenomatous polyposis coli (APC) gene, promoter sequence.	-48
U03019_p1	Melanoma growth stimulatory activity beta (MGSA beta) gene, partial cds	-590
U03254_p1	Acyl-CoA oxidase (AOX) gene, exon 1.	-379
U03735_p1	MAGE-3 antigen (MAGE-3) gene, complete cds.	462
U06078_p1	N-acetylgalactosamine 6-sulphatase (GALNS) gene, exon 1.	-243
U07172_p1	Insulinoma-associated (IA-1) gene, partial cds.	-314
U07663_p1	HB9 homeobox gene, exon 1.	-935
U07663_p1	HB9 homeobox gene, exon 1.	-474
U07802_p1	Tis11d gene, complete cds.	-565
U07802_p1	Tis11d gene, complete cds.	601
U07802_p1	Tis11d gene, complete cds.	1280
U08198_p1	Complement C8 gamma subunit precursor (C8G) gene, complete cds	862
U09360_p1	Intercellular adhesion molecule-1 gene, promoter region.	-669
U11239_p1	Cytosolic phospholipase A2 gene, promoter and exon 1.	457
U14939_p1	Folylpolyglutamate synthetase gene, partial cds.	221
U16271_p1	AMP deaminase isoform L (AMPD2) gene, exons 1A and 1B.	-820
U16271_p1	AMP deaminase isoform L (AMPD2) gene, exons 1A and 1B.	-587
U16271_p1	AMP deaminase isoform L (AMPD2) gene, exons 1A and 1B.	960
U17193_p1	Bax gene, 5' region.	203
U17193_p1	Bax gene, 5' region.	248

U19152_p1	P protein (P) gene, exon 1.	97
U19152_p1	P protein (P) gene, exon 1.	97
U20499_p1	Thermolabile phenol sulfotransferase (stm) gene, complete cds	687
U20734_p1	Transcription factor junB (junB) gene, 5' region and complete cds	741
U20734_p1	Transcription factor junB (junB) gene, 5' region and complete cds	2653
U21051_p1	G protein-coupled receptor (GPR4) gene, complete cds.	1314
U22364_p1	Cyclin B1 gene, promoter region.	-308
U25822	Heat shock protein 90 alpha	
U27317_p1	11 beta-hydroxysteroid dehydrogenase 2 (HSD11B2) gene, complete cds	-1256
U27317_p1	11 beta-hydroxysteroid dehydrogenase 2 (HSD11B2) gene, complete cds	-571
U28054_p1	Hepatocyte growth factor-like protein homolog gene, complete cds	-1725
U29201	Metastasis control (nm23-H1) gene, 5'-region.	260
U29201_p1	Metastasis control (nm23-H1) gene, 5'-region.	11
U29927_p1	AMP deaminase (AMPD3) gene, exon 1b and 1c and promoter	-975
U30787_p1	Uroporphyrinogen decarboxylase (URO-D) gene, complete cds.	925
U31120_p1	Interleukin-13 (IL-13) precursor gene, complete cds.	671
U32323_p1	Interleukin-11 receptor alpha chain gene, complete cds.	-358
U33446_p1	Prostasin gene, complete cds.	-368
U33453_p1	Protease nexin-1 (PN1) gene, promoter region.	-286
U33453_p1	Protease nexin-1 (PN1) gene, promoter region.	-1004
U33947_p1	Medium chain acyl CoA dehydrogenase (MCAD) gene, promoter region	-1312
U34070_p1	CCAAT/enhancer binding protein alpha gene, complete cds.	-268
U34859_p1	Tyrosine kinase (blk) gene, 5' flanking region and exon 1	-1242
U35052_p1	Helix-loop-helix protein (HEB) gene promoter region and exon1	-790
U37106_p1	Erythroid Kruppel-like factor EKLF gene, complete cds.	1129
U37106_p1	Erythroid Kruppel-like factor EKLF gene, complete cds.	1812
U41448_p1	Ribosomal protein S26 (RPS26) gene, complete cds.	330

U43140_p1	RhoG (ARH-G) gene, promoter region.	-848
U43748_p1	Frataxin (FRDA) gene, promoter region and exon 1.	1535
U47284	Cyclin D2	-1329
U47284	Cyclin D2	-1158
U47289_p1	Spasmolytic polypeptide (SP) gene, 5' region and exon 1.	-291
U50871_p1	Familial Alzheimer's disease (STM2) gene, complete cds.	-225
U50871_p1	Familial Alzheimer's disease (STM2) gene, complete cds.	-74
U52428_p1	Fatty acid synthase gene, partial cds.	-357
U52428_p1	Fatty acid synthase gene, partial cds.	-913
U55231_p1	Melanoma tumor antigen (Mart-1) promoter region	-714
U52694_p1	Creb-rp gene, exon 1 and partial cds.	-94
U56438_p1	Dioxin-inducible cytochrome P450 (CYP1B1) gene, complete cds.	-1680
U63108_p1	Eukaryotic initiation factor 4E (eIF4E) gene, promoter region and partial cds	-75
u63630	MCM4 and DNA-PKcs (pos. relative to MCM4)	820
U63721_p1	Elastin (ELN) gene, partial cds, and LIM-kinase (LIMK1) gene, complete cds	-875
U63721_p1	Elastin (ELN) gene, partial cds, and LIM-kinase (LIMK1) gene, complete cds	-883
U63833_p1	PAX6 gene, promoter region and exons 1 and 2.	-822
U64864_p1	PD-1 gene, promoter region and partial cds.	-68
U68093_p1	Poly(A)-binding protein (PABP) gene, promoter region and exon 1	-376
U71187_p1	Cholesteryl ester transfer protein (CETP) gene, partial cds and promoter region	-1014
U72648_p1	Alpha2-C4-adrenergic receptor gene, complete cds.	-566
U73167	Semaphorin V	6744
U73167	SM15 gene for IFRD2, interferon-related (PC4, TIS7 homologue)	199
U73167	SM15 gene for IFRD2, interferon-related (PC4, TIS7 homologue)	225
U75285	Survivin	7796
U76667_p1	Anion exchanger 2 (SLC4A2) gene, alternative promoters b1 and b2, exons 2, 1b1, 1b2 and 3 to 8	-472

U76667_p1	Anion exchanger 2 (SLC4A2) gene, alternative promoters b1 and b2, exons 2, 1b1, 1b2 and 3 to 8	-613
U76667_p1	Anion exchanger 2 (SLC4A2) gene, alternative promoters b1 and b2, exons 2, 1b1, 1b2 and 3 to 8	-580
U62290_p1	AE2 anion exchanger (SLC4A2) gene, promoter region and exon 1, complete sequence	-743
U77629_p1	Achaete-Scute homologue 2 (ASCL2) gene, complete cds.	562
U78027_p1	Bruton's tyrosine kinase (BTK), alpha-D-galactosidase A (GLA), L44-like ribosomal protein (L44L) and FTP3 (FTP3) genes complete cds	176
U78027_p1	Bruton's tyrosine kinase (BTK), alpha-D-galactosidase A (GLA), L44-like ribosomal protein (L44L) and FTP3 (FTP3) genes complete cds	483
U78027_p3	Bruton's tyrosine kinase (BTK), alpha-D-galactosidase A (GLA), L44-like ribosomal protein (L44L) and FTP3 (FTP3) genes complete cds	-254
U78027_p3	Bruton's tyrosine kinase (BTK), alpha-D-galactosidase A (GLA), L44-like ribosomal protein (L44L) and FTP3 (FTP3) genes complete cds	-221
U78073_p1	Polo like kinase (PLK) gene, promoter and partial cds.	-160
U79410_p1	Thrombospondin 2 (THBS2) gene, promoter region and exons 1A and 1B	650
u82618	Aspartylglucosaminidase promoter	promoter
U86758_p1	Netrin-2 like protein (NTN2L) gene, complete cds.	374
U86758_p1	Netrin-2 like protein (NTN2L) gene, complete cds.	-1446
U86758_p1	Netrin-2 like protein (NTN2L) gene, complete cds.	-521
U87926_p1	Aconitate hydratase (ACO2) gene, exon 1.	-139
U88317_p1	15-lipoxygenase gene, complete cds.	-295
U89323_p1	Interleukin-12 p40 gene, exon 1, complete sequence.	-955
U92868_p1	Reduced folate carrier (RFC1) gene, exons 1a, 1c and 1b	11
U93237_p1	Menin (MEN1) gene, complete cds.	-369
u96876	INSIG1 (insulin induced protein 1)	3218
V00488_p1	Alpha-globin germ line gene.	371
X03493_p1	c-sis proto-oncogene 5' region.	-252
X83706_p1	c-sis proto-oncogene (alternative exon-1a).	-208
X02158_p1	Erythropoietin.	382

X05006_p1	S-protein gene, complete cds. (Vitronectin)	-1097
AC011462, J04431, X05839	TGF beta gene (from AC011462, J04431, X05839)	-1018
AC011462, J04431, X05839	TGF beta gene (from AC011462, J04431, X05839)	2201
AC011462, J04431, X05839	TGF beta gene (from AC011462, J04431, X05839)	2779
X07056_p1	Alpha-amylase (EC 3.2.1.1) gene AMY2A 5-flank and exon 1.	-669
X07871_p1	CD2 gene exons 1 and 2 (and joined CDS).	-33
X12671_p1	Heterogeneous nuclear ribonucleoprotein (hnRNP) core protein A1	-442
X12671_p1	Heterogeneous nuclear ribonucleoprotein (hnRNP) core protein A1	-379
X12671_p1	Heterogeneous nuclear ribonucleoprotein (hnRNP) core protein A1	553
X13546_p1	HMG-17 gene for non-histone chromosomal protein HMG-17.	-104
X14940_p1	C mu gene for IgM heavy chain exons CH1-4, secretory.	-1225
x15723	Furin gene, exons 1 through 8.	-2326
X16287_p1	Alpha-enolase gene for non-neuronal enolase (EC 4.2.1.11) exon 1 (and joined mRNA)	-796
x51898	bcl-2 gene 5'-flanking region	promoter
X51952_p1	UCP gene for uncoupling protein exons 1 and 2.	-259
X52601_p1	hTOP1 gene for topoisomerase, 5'end.	-358
X54816_p1	Alpha-1-microglobulin-bikunin, exons 1-5 (encoding alpha-1-microglobulin, N-terminus.)	-563
X56997_p1	UbA52 gene coding for ubiquitin-52 amino acid fusion protein.	-145
X57928_p1	Prostatic secretory protein PSP-94, exon 1 and joined CDS	-294
X59964_p1	CST4 gene for Cystatin D.	-373
X60482_p1	H4/b gene for H4 histone.	452
X60484_p1	H4/e gene for H4 histone.	524
X61282_p1	N-RAS promoter region.	-46
X62654_p1	Me491/CD63 antigen.	-178
x62668	Melanotransferrin gene enhancer	
X67123_p1	TRAP gene for tartrate-resistant acid phosphatase type 5, exons 1-4	-144

X68969_p1	Betal integrin.	-940
X69118_p1	Promoter sequence of MUC1 gene.	-1570
X70286_p1	Thioredoxin, exon 1.	-423
X72735_p1	Arylsulfatase B, exon 1.	-868
X74107_p1	Antigen of the monoclonal antibody Ki-67.	1578
X74840_p1	Wilms tumor (WT1) gene promoter.	213
x74961	ACPP gene for prostatic acid phosphatase	-2034
X77491_p1	G11 exon 5,6,7 and gene for C4A.	-77
X82032_p1	B-myb gene.	-309
X82201_p1	RPL19 gene.	-261
X82245_p1	Nidogen gene (exon 1).	-1893
X82245_p1	Nidogen gene (exon 1).	1138
X87344_p14	DMB gene	-1988
X87344_p15	DMB gene	-1178
X94359_p1	5' region of angiotensin-I converting enzyme.	-1177
X94359_p1	5' region of angiotensin-I converting enzyme.	-861
X94563_p1	Dbi/acbp gene exon 1 & 2.	-123
X95151_p1	Brca2 gene exon 1.	-19
X95536_p1	Ear1 gene.	-471
X98053_p1	G13 gene.	-109
Y00067_p1	Neurofilament subunit M (NF-M).	-134
Y00371_p1	Hsc70 gene for 71 kd heat shock cognate protein.	1666
Y00371_p1	Hsc70 gene for 71 kd heat shock cognate protein.	558
Y07558_p1	PILOT gene, 5' flanking region.	-846
Y07712_p1	ATFa gene, promoter region.	-201
Y08733_p1	MIIX gene, promoter region.	-924
y09540	AHSG gene, partial.	-3072
Y10284_p1	TRAF1 gene, putative promoter region.	-485
Y12773_p1	TRIDENT/HFH11 gene, promoter sequence.	-113
Y13901_p1	FGFR-4 gene.	629

Y15082_p1	p14.5 gene, partial exon 1 and promoter; hPOP1 gene exon 1 and promoter and bidirectional promoter region	-167
Z19561_p1	FAH gene promoter region.	-554
Z21818_p1	Carcinoembryonic antigen gene.	-41
Z23091_p1	GPV gene encoding platelet glycoprotein V precursor.	-787
Z26491_p1	Catechol O-methyltransferase.	34
Z29077_p1	Cdc25 gene promoter region.	-659
Z29078_p1	Cyclin D1 gene promoter region.	-552
Z84811_p1	Muscle nicotinic acetylcholine receptor gene promoter.	-740

[0110] Table 2 discloses nucleic acids, genes, or polypeptides from Table 1, for use in the claimed methods.

Table 2. Accession number **Gene definition**

AF009247_p1	CLCN6
AF148461_i1; NP_001284; P54105	CLNS1A
u76667_p1	Anion exchanger 2 (SLC4A2)
AF255613_p1	Teratoma-associated tyrosine kinase (TAPK) (or gklp)
U86758_p1	Netrin-2 like protein (NTN2L) gene, complete cds.

III. Computer search for genomic sites for use in ChIP assays.

[0111] Computer searches were performed according to Wang, *et al.* (2001) *J. Biol. Chem.* 276:43604-43610. The search encompassed a population of 6541 Genbank entries. Human genomic sequences were searched for an E box motif, located with a distance of 2 kb on either side of transcription start sites (Abstract of Mol, *et al.* (1995) *Mol. Cell. Biol.* 15:6999-7009). 1630 loci scored positive, with one or more E-boxes within the predetermined boundaries, identifying 2224 E-boxes. 93 additional loci were included in the screen, of which 69 E-boxes were outside the +/- 2 kb boundary.

IV. ChIP assays.

[0112] ChIP assays were performed as described in Frank, *et al.* (2001) *supra*, with the following modifications. Fixed cells (1.5-3.3 x 10^8 cells) were sonicated in 6 ml of SDS buffer. The lysate was diluted with 3 ml of Triton Dilution Buffer (100 mM Tris, pH 8.6, 100 mM NaCl, 5 mM EDTA, 5.0% Triton® X-100). Immunoprecipitation was performed using 9 ml of lysate, and either 0.05 mg polyclonal anti-c-Myc antibody N-262 (cat. no. SC764; Santa Cruz Biotechnology, Santa Cruz, CA) or 0.5 ml of blocked protein A beads, i.e., a 50% slurry of Protein A-Sepharose ® (Amersham Biosciences, Piscataway, NJ), per sample. For large scale experiments, DNA preparations from three independent ChIPs were pooled and diluted in 6 ml of water.

[0113] Polymerase chain reactions (PCR) were performed with 0.004 ml of DNA and 800 nM primers, diluted in a final volume of 0.02 ml in SYBR Green Reaction Mix (Applied Biosystems, Foster City, CA). SYBR ® Green PCR Core Reagents (Applied Biosystems, Foster City, CA) was used to monitor PCR product.

[0114] Control immunoprecipitations were performed in a variety of ways, e.g., by using pre-immune serum rather than by using anti-Myc antibody, or by using Myc-deficient cells with the standard ChIP procedure.

[0115] ChIP assays on a large number of E box target sites were conducted on chromatin from five different cell lines, U-937 cells, HL60 cells, P493-6 cells, T98G cells, and WS1 cells. Chromatin from U-937 cells were used for two types of tests, ChIP assays where the targets were E boxes associated with a promoter, and ChIP assays where the 134 E boxes were randomly chosen from chromosome 21, i.e., not necessarily associated with a promoter. Chromatin from the other cell lines were subjected to ChIP assays targeting only E boxes associated with a promoter.

[0116] The results from the U937 cells were as follows. 809 E-boxes/genes were selected from a list of 2224 E-boxes for use in ChIP assays. A computer screen of U937 cells and HL60 cells identified 351 promoter-associated sites, and these sites were used in ChIP assays of U937 cells. An additional 458 sites were tested in U937 cells, where these additional sites were selected according to biological interest, resulting in a total of 809 sites tested in the U937 cells. ChIP assays were applied to these 809 target sites were conducted with anti-Myc antibody (experimentals) and without anti-Myc antibody (controls). Myc bound to 336 (42%) of the 809 sites, i.e., there were about 340 positive screening sites. Recovery for the control assays ranged from about 0.01% input to about 0.06% input, while recovery for the experimental assays yielded recovery data ranging from a recovery of about 0.02% input to a recovery of about 2.0% input. The positive-screening sites included E-boxes/genes from, e.g., NUC, HSP10/60, CAD, TERT, GPAT/AIRC, and cyclin D2.

[0117] U937 cells were also used for a separate study that served as a control study. A number of randomly occurring E-boxes were analyzed, that is, E-boxes not necessarily associated with promoters. 134 Randomly occurring E-boxes in chromosome 21 of U-937 cells were subjected to ChIP assays. Myc bound to five of these sites (3.7%) at relatively low levels

though none screened positive by the above-stated criteria. None of the target E-boxes were bound at high levels. In most cases, recovery of the targeted gene for both control and experimental ChIP immunoprecipitations ranged from only about 0.01% to only about 0.08%.

[0118] HL60 cell results were as follows. In studies with chromatin from HL60 cells, 125 (36%) of the 351 promoter-associated E-boxes/genes tested screened positive in the ChIP assays.

[0119] P493-6 cell line results were as follows. These cells allow repression of a c-Myc transgene by tetracycline (Tet), resulting in G1 arrest in the presence of serum. Subsequent removal of tetracycline induces Myc, and re-entry into the cell cycle (Schuhmacher, *et al.* (1999) *Curr. Biol.* 9:1255-1258; Schuhmacher, *et al.* (2001) *Nucl. Acids Res.* 29:397-406). The sources of cells in the following ChIP assays were Tet-treated cell preparations, where Myc was repressed, and Tet-removed-cell preparations to allow induction of Myc (culture for 8 h after removal of Tet).

[0120] ChIP immunoprecipitation assays on the same collection of target E boxes/genes were conducted under three different conditions: (1) With anti-Myc antibody (no Tet); (2) With anti-Myc antibody (plus Tet); and (3) Control without anti-Myc antibody (no Tet).

[0121] The highest signals were from ChIP assays using anti-Myc antibodies, where cells had been induced to synthesize Myc. The recovery for most of the E boxes/genes in this test was above 0.1% input. Intermediate results were produced by ChIP assays containing anti-Myc antibodies, performed on non-induced cells. The recovery for most of the E/boxes/genes in this test was below 0.1 % input. Control ChIP assays without anti-Myc antibody using non-induced cells showed relatively low signals. The recovery for most of the E boxes/genes in this test was below 0.04% input. Upon induction of Myc, 330 of the 388 (85%) tested E-boxes/genes tested positive. Since this enrichment was dependent on removal of tetracycline, it demonstrated that a positive signal in the ChIP assay was dependent on increased concentrations of intracellular Myc protein.

[0122] Human glioblastoma cells (T98G) were studied. This cell line was pre-treated for four hours with serum before use in ChIP assays. Experimental assay mixtures containing anti-Myc antibody and control assay mixtures without anti-Myc antibody were assembled, and recovery for each E-box/gene was expressed as percent input. The ChiP assay signal was greater

for the experimental ChIP assays than for the control ChIP assays, for nearly all of the E boxes/genes tested. Recovery of the target genes, for most of the experimental ChIP assays, ranged from about 0.008% input to about 0.8% input, while the corresponding values for control ChIP assays ranged from only about 0.001% input to only about 0.02% input.

[0123] Primary human fibroblasts (WS1) were studied. The cell line was pre-treated for four hours with serum before use in ChIP assays. Experimental ChIP assays containing anti-Myc antibody and control ChIP assays without anti-Myc antibody were conducted. Recovery for each E-box/gene was expressed as percent input for the experimental and control assays. The results demonstrated that Myc binding resulted in a signal above control for about half of the genes tested in serum-treated WS1 cells.

[0124] Following collection of the ChIP data from the various cell lines, the data from specific pairs of different cell lines were compared to each other, e.g., by comparing results from a particular E box/gene from HL60 cells with the results from that same gene from U937 cells. The pairwise comparisons were made for all of the E boxes/genes tested that were common to both cell lines.

[0125] The goal was to determine if Myc bound to overlapping populations of target sites in various cell lines. The comparisons, i.e., pairwise plots, disclosed ChIP data from U937 cells versus from HL60 cells; U937 cells versus P493-6 cells; T98G cells versus U937 cells; and WS1 cells versus U937 cells. In all combinations, most of the high-affinity sites clustered together, as did the low affinity sites, resulting in a roughly linear continuum. In other words, the ChIP signal (% input), for any given gene, was roughly comparable in tests among the different cell lines. As stated above, the resulting plots were roughly linear. Thus, the relative Myc-binding efficiencies of promoter E-boxes was conserved among different cell lines.

[0126] In all pairwise combinations, there were a minority of outliers, that is, sites that were bound efficiently only in a given cell line. These differences might be due to tissue-specific accessibility of chromatin or to exclusion of Myc binding through *de novo* methylation of selected CpG islands.

V. Cellular levels of Myc protein.

[0127] Myc protein was measured in HL60, U937 cells, Raji cells (Raji Burkitt lymphoma), P493-6 cells, T98G cells, and WS1 cells using western blot analysis. For each blot, 50 micrograms of whole cell lysate from exponentially growing cells was separated by SDS PAGE and probed using a monoclonal anti-Myc antibody (9E10). HL-60 and WS1 cells expressed low levels of Myc, while Raji cells and induced P496-3 cells, contained relatively high levels of Myc. U-937 cells and T98G cells expressed intermediate amounts of Myc protein, where expression by U-937 cells was greater than for the T98 cells. The range of Myc protein levels in these cell lines covered about two orders of magnitude.

[0128] Myc was also measured in P496-3 cells over the course of time, with induction of Myc by removal of tetracycline (Tet) and measurement at 0, 1, 3, 6, 9, and 12 h after induction by Tet removal. The results demonstrated no detectable expression at t = 0 h, slight expression at t = 1 h, with about 50% maximal expression occurring at 3h and 6h, and maximal expression at 9 h and 12 h.

[0129] These results, taken with those comparing ChIP signals acquired from tests of the different cell lines, demonstrate that the distribution of binding to various Myc targets remains approximately the same, even where intracellular levels of Myc differ widely.

VI. Correlations between Myc-binding and induction of mRNA expression.

[0130] Time courses for induction of a number of genes in P493-6 cells and in T98G cells were studied, where induction was by Myc-induction (P493-6 cells) or by serum induction (T98 cells). mRNA encoding rpP0, NUC, DKc1, CASP8, AMPD2, and C-MET was measured at t = 0, 1, 3, 6, 9, and 12 hours, in both types of cells.

[0131] With P493-6 cells, maximal or near-maximal induction of NUC (7-fold induction) and DKC1 (6-fold) appeared at 6-12 h after Tet removal. Little or no increase in mRNA levels were detected for message expressed from rpPO, CASP8, AMPD2, or c-MET genes. In all cases, little or no change in gene expression was found in control incubations where continued presence of Tet prevented induction of Myc.

[0132] With T98G cells, maximal induction of NUC (6-fold), DKC1 (14-fold), and C-MET (10-fold) occurred at about 9 hours. RpP0 was gradually induced, over the course of six

hours, to a maximum of about 2.5-fold, where maximal induction was found at 6-12 hours. There was little or no detectable induction of CASP8 and AMPD2 during the 12 hour incubation period.

[0133] The possible correlation between ChiP assay % input and fold-induction of mRNA expression was studied in P493-6 cells, where 75 genes were examined, and for T98 cells, where 37 genes were examined. Although there was a correlation between ChIP signal and fold-mRNA induction for some genes, there was little overall correlation between ChIP signals and fold-mRNA induction for the genes that were tested. Thus, for some genes Myc binding alone can be sufficient to provoke increases in gene expression, while for other genes factors in addition to Myc binding may be required from gene expression.

VII. Conditions of cell culture.

[0134] U937 and HL60 cells were grown in RPMI (Roswell Park Memorial Institute) medium supplemented with 10% fetal calf serum. For analysis by the ChIP technique, 1.5 liters of exponentially growing cells were diluted to 2-3 x 10^5 cells/ml one day before harvesting. P-496-3 cells are described (Kempkes, *et al.* (1995) *EMBO J.* 14:88-96). P496-3 cells were grown in RPMI medium supplemented with 10% fetal calf serum, NEAA (BioWhittaker, Inc., Walkersville, MD), and 2 mM L-glutamine (BioWhittaker, Inc.). Repression and re-expression of Myc was according to Schuhmacher, *et al.* (2001) *Nucl. Acids Res.* 29:397-406. For ChIP, 2 liters of exponentially growing cells were diluted to 3 x 10^5 cells/ml and tetracycline (0.0001 mg/ml) (Sigma-Aldrich, St. Louis, MO) was added for 72 h. To re-induce expression of Myc, cells were washed three times in prewarmed RPMI medium containing 10% fetal calf serum before culturing for the indicated period of time.

[0135] T98G and WS1 were from American Type Culture Collection (Manassas, VA) and grown in D-MEM supplemented with 10% fetal calf serum. Cells were rendered quiescent by growth to confluent density, followed by incubation for three days in serum-free medium. To induce cell cycle entry, cells were harvested by trypsinization and re-seeded 1:4 onto plates containing D-MEM/10% FCS. For ChIP assays, cells from 15 confluent 150 mm dishes, or the equivalent amount of cells, following dilution (splitting) were used. One confluent plate, or the equivalent amount of cells, were used for RNA extraction.

[0136] Many modifications and variations of this invention, as will be apparent to one of ordinary skill in the art can be made to adapt to a particular situation, material, composition of matter, process, process step or steps, to preserve the objective, spirit, and scope of the invention. All such modifications are intended to be within the scope of the claims appended hereto without departing from the spirit and scope of the invention. The specific embodiments described herein are offered by way of example only, and the invention is to be limited by the terms of the appended claims, along with the full scope of the equivalents to which such claims are entitled; and the invention is not to be limited by the specific embodiments that have been presented herein by way of example.